Melatonin Receptor A Gene, Melatonin Receptor C Gene and Ovocalyxin-32 gene polymorphisms associated with some Egg Production in Local Iraqi Chicken

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Abstract

The aim of this research is to detect genetic polymorphisms of some candidate genes associated with egg production trait and to find the relationship between some of these genetic sites and the productive traits of Local Iraqi chickens that will be used for the national poultry breeding programs to increase quality egg production in endogenous chicken. Genomic DNA were extracted and then amplified three genes (melatonin receptor a gene MTNRa, melatonin receptor c gene MTNRc and ovocalyxin-32 gene (OCX-32). (MTNRa) gene amplified by PCR-SSCP method. The distribution of percentages in female Iraqi Local chicken studied for (MTNRa) were 73, 20, 5 and 2% for TN, TNA, NN and TT SSCP-genotype respectively. It turned out that phenotypic polymorphisms of MTNRa gene was significant ($p<0.05$) with Serum Melatonin and Magnesium concentrations, in addition to First egg weight FEW and Body weight at maturity. (MTNRc) gene amplified by PCR-RFLP method, the distribution of percentages of MTNRc genetic location in female Iraqi Local chicken Flock studied were 23, 61 and 16 % for MM, Mm, mm RFLP-genotype respectively, it turned out that phenotypic diversity of MTNRc gene was significant ($p<0.05$) in mean of Serum Melatonin and Magnesium concentration, in addition to First egg weight FEW and Body weight at maturity. (OCX-32) gene amplified by PCR-SSCP method. The distribution of percentages in female Iraqi Local chicken studied were 98 and 2% for WS and SS (SSCP-genotype) respectively. It turned out that phenotypic polymorphisms of OCX-32 genetic site were significant ($p<0.05$) with serum Melatonin and Magnesium concentration and Body weight at maturity. The present research suggests that the genes MTNRa, MTNRc and OCX-32 could be used as a possible marker in Marker Assisted Selection (MAS) and genomic selection programs for local Iraqi chickens.

Key words: *Local Iraqi chicken, genetic polymorphism, Melatonin receptor c, egg production traits, Local chicken, crossbreeding, breeding poultry and improvement, Melatonin receptor a gene, ovocalyxin -32 gene, OCX-32, MTNRa*

Introduction

Local Iraqi 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 egg weight and egg mass, in addition, it was found that they classified as a good performance for egg production[1], 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. It was strongly needed to establish breeding programs that allows improving performance of local chickens[2]. 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. 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, and because of modern trends in the use of molecular genetics technologies for genetic improving in the selection programs[3]. Egg production is a polygenic inheritance trait with low to moderate heritability, which depends on the period-involved[4]. Multi traits selection to improve fitness and simultaneously increase egg yield is therefore difficult to accomplish by traditional, direct phenotypic selection. Thus, selecting individuals with additional information on their genotype for markers associated with QTLs for fitness and reproduction (marker-assisted selection, MAS) is preferred[5] Molecular markers were used to map QTLs related to chicken growth and reproduction such as body weight (BW), Egg number (EN), and Egg Mass (EM) in the past decade[6]. So, the using of molecular marker in breeding has a huge advantage, for the time being, molecular markers are widely used in poultry breeding[7], thus, the aim of the present paper was to detect genotype polymorphism for egg production traits genes of local Iraqi chickens.

Materials and Methods

The experiment was 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, it has conducted on 100 Local Iraqi chickens raised at an experimental farm, where all hens were individually kept in cages. The hens in the period of laying eggs (28-47 weeks old) were mated with male Iraqi chicken at ratio of 1 cock: 10 hens.

Genotypes of candidate genes were determined by PCR-RFLP and PCR-SSCP techniques. extraction and purification of DNA from hen's blood[8], amplification of DNA by using appropriate primers corresponding to each gene and determination of the genotype by electrophoresis band on 2% agarose gel. Details of the primer sequences, annealing temperature, and other details are presented in Table1.

Table 1 Sequences of PCR primers for genomic DNA

Genetic position	accession number	Primer sequence (5` – 3`)	Chr. number	Length (bp)	Annealing temperature (C°)	Reference
MTNRc SNP	NC_006091	(F): GGTGTATCCGTATCCTCTAA (R): GACAGTGGGACAATGAAGT	4	372	52	Li, 2013[6]
MTNRa mRNA	NM_205362.1	(F): CCTGGCAATTGCAGACTTGG (R): TTCTGGGTCAACAGCCACAG	4	595	63.1	Designing genes, using NCBI BLAST method
OCX-32	AM076826.1	(F): TGCAGTCAAGTAAATCAGGGTAGA (R): ACTAGCAAAGTTTTTCAGTAGGTCC	9	589	60	

PCR-RFLP and PCR-SSCP Analysis

An *MboI* recognition site was created by the MTNRc SNP. The PCR products of this locus were digested with the restriction enzyme *MboI* 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 MTNRc amplicon. PCR-SSCP analysis of the MTNRa and OCX-32 SNP was performed according to Sivaraman[9]

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≤ 0.05) was considered statistically significant. SPSS, Statistical Analysis System, Version24, 2019. Significance of the least squares means was tested with the Duncan's Multiple Range test.

Results

The present study includes (100) samples as local Iraqi chicken's flock. 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.

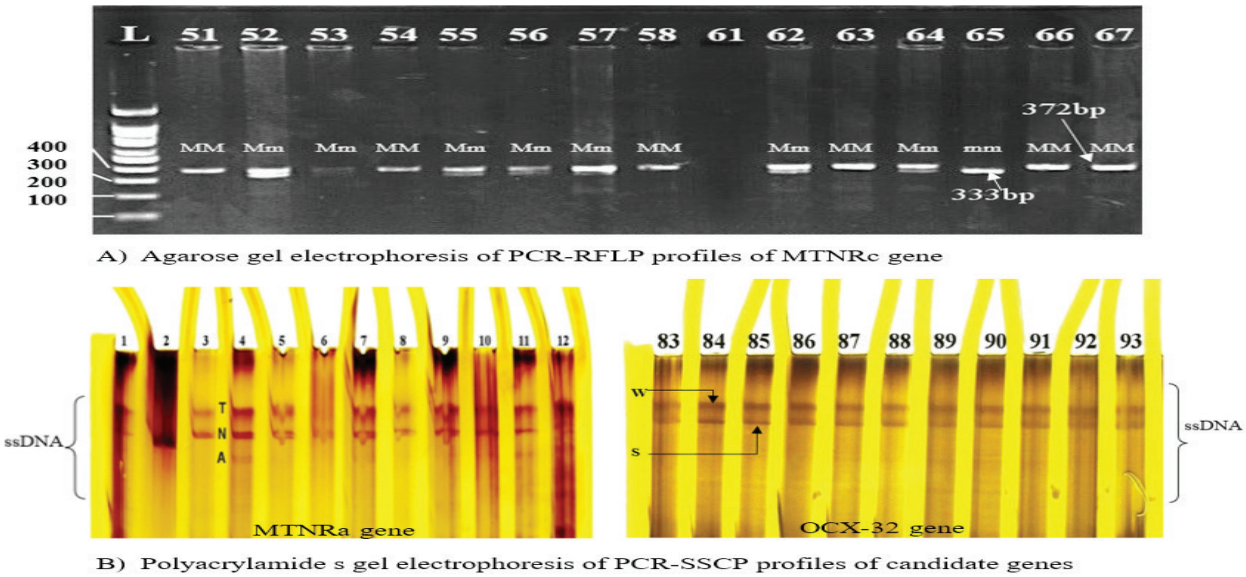

Figure 1: Gel electrophoresis of (A) PCR-RFLP and (B) PCR-SSCP profiles of candidate genes.

Figure 1 (A) shows the electrophoresis results of PCR products that were digested with *MboI* restriction enzyme for MTNRc gene and Figure 1 (B) shows the electrophoresis results of amplification of the target site of conformational polymorphism of the Melatonin receptor a and OCX-32 genes using PCR-SSCP method.

Table 2 Allele and genotype frequencies of genes in Local Iraqi chicken.

Genes	Genotype Percentage (%)				Allele			*HWE
MTNRa	TT	NN	TNA	TN	T	N	A	P≤0.01
	2%	5%	20%	73%	95%	97%	20%	
MTNRc	MM	Mm	mm		M	m		P≤0.01
	23%	61%	16%		0.54	0.47		
OCX-32	WS	SS			W	S		P≤0.01
	98%	2%			0.49	0.51		

*HWE: Hardy–Weinberg Equilibrium; significant differences (P≤ 0.01)

Table 3 polymorphism relationship of local chickens for some Physiological and egg production parameters.

Genes	Traits genotype (M± SE)	Serum Melatonin Hormone (pg/ml)	Serum Magnesium (mg/dl)	First egg weight (FEW)(gm)	body weight at maturity (BWM) (gm)
MTNRc	MM	132.62±19.378 a	2.96±0.067 a	34.14±0.93 a	1471±41.20 a
	Mm	92.77±5.046 b	3.23±0.102 a	32.68±0.513 ab	1324±20.32 b
	mm	100.28±8.241 b	2.5±0.242 b	31.23±0.902 b	1382±62.63 ab
MTNRa	TT	90.83±0.02 b	3.6±0.01 a	29.7±0.03 b	1108±0.04 b
	NN	120.46±12.865 a	2.1±0.4 b	35.34±0.968 a	1283±70.68 ab
	TN	106.61±7.392 ab	3.13±0.081 a	32.64±0.509 ab	1390±24.361 a
	TNA	92.47±7.78 ab	2.97±0.216 ab	33±0.792 ab	1332±24.279 ab
OCX-32	WS	103.97±5.82	3.043±0.08	32.81±2.419	1332.69±25.651
	SS	62.3±0.01	3.7±0.01	31.35±0.35	1201.25±23.67
	Sig.	0.000	0.000	NS	0.000

M: Mean; SE: Standard error; different letters per class indicates significant differences (P≤ 0.05); NS: non-significant

The detection of allelic and genotypic frequency plays an important role in animal breed selection. Based on these frequencies, assumption and their association with phenotype traits would support to create new individuals with desirable genotype and phenotype. The frequencies of MTNRa, MTNRc and OCX-32 alleles and genotypes are shown in Table 2 and the Genetic and Phenotypic correlations of candidate genes showed in Table3. At the MTNRa polymorphic locus, N allele showed higher frequency than T and A alleles and accounted for 97% in the population. Moreover, the appearance of T allele was higher than A resulting in highest percentage of TN genotype in Local Iraqi chickens (73%). The analysis of MTNRc which restricted by *MboI* restriction enzyme, 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 and when followed Allele frequency for MTNRc gene in Table 2 showed 0.54 for M allele and 0.47 for m allele. the percentages of phenotypic occurrences of the exon OCX32 gene in local Iraqi chicken showed high significant differences ($p<0.05$) for the different genotypes, which amounted to 98% and 2% of the genotypes are WS and SS respectively, meaning that there is a clear prevalence of

the heterozygous formations carrying the WS genotype, followed by rarely found the pure homozygous SS genotype and the frequency ratios for alleles were (98%) W haplotype allele and (100%) for S haplotype allele In addition to the disappearance of the genotype WW. When corelated relationship of the polymorphism of MTNRa gene on some of the physiological parameters of important PCR-SSCP genotypes, it was found a significant difference ($p \leq 0.05$) with (melatonin and magnesium) and from the significant value of Serum concentrations of melatonin 120.46 (pg / ml) for the NN genotype, and were set a significant at 3.6 and 3.1 (mg/dl) magnesium concentration for TT and TN, respectively, there was a significant difference among genotypes with FEW and BWM, significant value for FEW (35.34 gm.) at NN genotype and BWM (1390.02gm.) at TN genotype. Correlation polymorphism of MTNRc gene on Melatonin (pg/ml) and magnesium (mg /dl) serum concentrations record a significant difference ($p \leq 0.05$) and the highest value recorded by MM genotype, and the results found that there is a significant difference ($p \leq 0.05$) by the mean weight of the first egg (FEW) 34.14 gm. and body weight at sexual maturity (BWM)1471.04 gm. for the genotype (MM). The corelated relationship of the polymorphism of OCX-32 gene on some of the physiological parameters of important PCR-SSCP genotypes, it was found that there was a significant difference for physiological parameters (melatonin, and magnesium) and from the value of Average serum concentrations of melatonin for the WS and SS genotypes were set at 103.97 pg/ml and 62.3 pg/ml respectively, serum concentrations of magnesium for the WS and SS genotypes were set at 3.043 mg/dl and 3.7 mg/dl respectively, egg production trait polymorphism relationship results found that the mean weight of the first egg (FEW) for the genotypes (WS, SS) was 32.81and 31.35 grams, respectively, and since no significant differences between the genotypes were identified but for body weight at sexual maturity (BWM), it became apparent that they recorded significantly the highest value ($P<0.05$) (1332.69) gm. For WS genotype, while the lowest value of 1201.25 gm was recorded by the genotype (SS)

Discussion

Egg production is an important economic trait in the poultry industry[5]. Our findings confirm significant impact of melatonin receptor a (MTNRa) on female reproductive traits (FEW and BWM) in addition to significant difference ($p \leq 0.05$) with serum Melatonin hormone (pg/ml) and serum Mg(mg/dl) concentration in local Iraqi chicken, some of these results similar to Li[10] which studied association of three melatonin receptor genes with reproductive traits in Erlang Mountain chicken and reported that chickens at MTNRa studied by sequencing produced their first eggs earlier with genotype EE (but, perhaps consequentially, eggs of lower weight). In poultry breeding programs, egg number at 300 days of age (EN) is used as the most valuable indicator of total egg production potential. Recently, many researchers have sought correlations between markers of candidate genes and reproductive traits in chickens [11; 12; 13]. The MTNRa gene studied in this study are located on chromosome 4 to which highly significant QTL effects on production traits have been mapped in previous studies[14;15;10]. Interestingly, the expression of the MTNRa mRNA has been found lowest level in small yellow follicle and increased in the granulosa layer of the chickens' ovarian follicles[16] but relationship between MTNRa gene polymorphisms and seasonal reproduction activity associated with melatonin concentration is what influences the ovarian activity leading to its effect on the resulting eggs and egg production in general[17]. The search for molecular markers that influence reproductive traits of chickens has been well reported by[18;15; 19; 20]. Ommeh[21] identified polymorphisms in the GnRHR (gonadotropin-releasing hormone receptor) and NPY (neuropeptide Y) genes but discovered no association of the two polymorphisms with total egg production. Li[10] was found specified polymorphisms a statistically significant association of the MTNRa with EN, demonstrating that animals with the FF genotype produce their first egg at a lower age (AM) and produce more eggs at 100 days of age after maturity (EN) than those with the EE, EF ($P < 0.05$). AL-Rekabi[2] concluded that, there is a clear significant difference for phenotypic polymorphism of the genotypes of the growth hormone (GH) gene and its receptor (GHR) on quality and egg production traits in local Iraqi chickens. in present study a statistically significant association of the N allele in both studied flocks, suggesting strong balancing selection (overdominance). Previous studies have found that the GnRH-I gene, vasoactive intestinal polypeptide receptor-1 (VIPR-1) gene[22],

follicle-stimulating hormone receptor (FSHR) gene[18], significantly affects age maturity or broodiness. In the current study we have shown that allele N in Local Iraqi chicken for MTNRa gene are associated with (BWM, Melatonin Hormone, Magnesium and FEW). The Hardy-Weinberg equilibrium (HWE) is the primary starting point for all genetic investigations of populations, whether the goal is to discover or estimate the effects of all forces disrupting HWE[23]. Determining the presence or absence of a deviation from it is important, because it allows inferences regarding the effects of selection or other factors that shape the population composition (i.e., the general trend of animal husbandry can be traced by Hardy Weinberg equilibrium) and then form the study of the relationship between different allelic variants of the target genes Relevant economic features of different strains or productivity trends are the next step to explore the success of its purpose[24]. In our current study, it is important to continue the resulting flock subjecting to Hardy Weinberg equilibrium because the purpose of this research maintaining the traits of local Iraqi chickens that Distinguishing, (like a high viability to adapt to the prevailing environmental conditions such as their heat resistance and their needed a simple diet, especially in the free-range system and rural breeding) subject to Hardy Weinberg equilibrium[1] Egg production is a complex process that not only involves the reproductive system but also depends on the availability of specific nutrients and the efficiency of their utilization. For example, melatonin, enhance the egg laying productivity of hens. Higher serum melatonin levels in hens associated with more egg production, increasing promoted the maturation and development of oocytes[25]. There are few reports that are related to the effects of melatonin application on the yield of egg production in hens, the egg-laying rate was positively associated with blood melatonin levels. Furthermore, melatonin implantation at a dose of 10 mg significantly improved their egg-laying rate. In addition to the increase in the egg-laying rate, the total egg weight, and also improved the quality of the eggs[26]. We found there was a significant increase in the concentration of melatonin hormone with the genotype MM for MTNRc gene Table (3), which means MM genotype of the MTNRc gene is associated with the significant increase serum melatonin hormone. our results similarly recent studies 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 [6; 22]. The organic matrix proteins involved in eggshell formation and the important role of *OCX–32* during the termination phase of eggshell formation. Moreover, *OCX–32* expression levels were related with Quality and Egg Production Traits[27]. There are several reports in the literature of variation within this gene and subsequent examination of its influence on various shell quality traits[27;28;29]. The results of our study similar to [28]Takahashi,2010 which identified haplotypes of OCX32 utilizing three SNPs within exons 2, 3, and 4 in an F2 generation of a line cross. In view of the extreme filtering applied to the consistency traits of the eggs in local Iraqi chicken, this tends to be a very large number. Maybe the heterozygosity of the OCX32 protein confers a selective benefit, thus leading to the preservation of genetic diversity in the OCX32 gene[29]. From the results Ovogalyxin-32 gene Table (3) in this gene we found that there is a highly significant difference $p \leq 0.05$ with serum Melatonin hormone, serum Magnesium and Body weight at maturity that mean this gene closely related with these phenotypes (PHTs). As the ovocalyxin–32 SNPs are located in gene coding regions with nonsynonymous substitutions of the protein sequences as deposited in the DDBJ database, the association between haplotypes and these parameters suggests that these substitutions may be directly responsible for these phenotypic alterations[28]. Fulton[29] conclude that the presence of multiple PHTs has consequences for the application of whole genome selection and high-density SNP analysis in which SNPs are assumed to be in high LD with all surrounding variants. This is correct if only two haplotypes occur within a line but erroneous if multiple haplotypes occur. The haplotype analysis of OCX32 presented here clearly showed that just two haplotypes occur are common in local Iraqi hens, it is plausible that the statistically significant linear trends manifested by PHTs in local Iraqi chickens which reflected ongoing selection targeted to the chromosomal region surrounding the OCX32 gene in the studied flock. The wide variation in some physiological parameters

between species of birds may be due to the circadian rhythms, the effects of diet, gender, and age. Like we assume that the increase in the serum magnesium level was due to increased biosynthesis and accumulation in the egg yolk[30]

Conclusions

1- Local Iraqi chicken responsive to the Hardy Weinberg equilibrium law for MTNRc genetic location by PCR-RFLP.

2- Phenotypic diversity used by PCR/RFLP of MTNRc gene was significant ($p<0.05$) in mean of melatonin concentration, body weight at sextual maturity, serum Mg conc., FEW.

3-Phenotypic polymorphisms of MTNRa gene were significant ($p<0.05$) with serum Magnesium concentration, First Egg Weight, body weight at sextual maturity, and serum melatonin hormone.

4- phenotypic polymorphisms of OCX-32 gene was significant ($p<0.05$) with serum melatonin hormone, Mg concentration and BWM.

5- the genes MTNRa, MTNRc and OCX-32 could be used as a possible marker in Marker Assisted Selection (MAS) and genomic selection programs.

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