Polimorphic Explore of Esr1, Esr2 and Foxl2 Genes and Interaction Effect of Esr1 and Foxl2 with Productive Traits of Brown Local Iraqi Chickens

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Abstract

The estrogen receptor 1 (ESR1) and estrogen receptor 2 (ESR2) plays a crucial role in the growth and development of follicles and ovulation in chickens. Fork-head box L2 (FOX L2) gene and its protein is active in several tissues, such as the ovaries, it has a pivotal role in ovarian development and function, like regulation of egg formation in poultry. This study aimed to detect the polymorphism of ESR1, ESR2 and, FOXL2 genes and investigate interaction between ESR1 and FOXL2 in the productive performance of brown local Iraqi chickens (BLIC). A total of 104 BLIC, at 17 weeks of age. Four novel single nucleotide polymorphism (SNPs). Every polymorphism a number had been given according to its location within gene, one of them in ESR1 (T57198C) intron 3 with three genotypes: Wild(TT), Heterozygous(TC), and Homozygous(CC), and two SNPs within ESR2 were assessed, one of the identified SNPs T39565C transition located within intron 7 with two alleles(T and C) and three genotypes were found: Wild(TT), Heterozygous(TC), and Homozygous(CC), another SNP G40100A transition within (3 prime UTR), with two alleles (G and A) and three genotypes were found: Wild(GG), Heterozygous(GA) and Homozygous(AA) and the SNP in FOXL2 (C919T) gene with two genotypes: Wild(CC) and Heterozygous(CT) encoding region were identified through PCR-DNA sequencing. An interaction between the ESR1 and FOXL2 genes has been proposed, and six haplotypes (H1=TTCC, H2=TTCT, H3=TCCC, H4=TCCT, H5=CCCC, H6=CCCT) were obtained due to interaction between these two SNPs. Chi-square analysis showed no significant in genotypic and allelic frequencies for each SNP which revealed those genes were agreement with Hardy-Weinberg equilibrium. Association analysis of haplotypes with production traits revealed that individuals have H4 genotype achieved higher body weight at sexual maturity, at 60 weeks of age and egg weight at 45 and 60 weeks of age, whereas, the higher number of eggs were exhibited to individual with H6 from onset egg till 60 weeks of age. The two genotypes; TCCT and CCCT showed better combination than others with respect to production performance. In conclusion, our findings provided new evidence that the two SNPs (ESR1 and FOXL12) with special interaction may have potential effects on productive traits and beneficial effects in laying breeding programs.

Keywords: Genes, Egg production, Foxl2.

1. Introduction

Egg production is a vital process regulated by hypothalamic - pituitary gland - a wide range of endocrine system pathways through a set of complex genes control of egg production traits, therefore, it is very difficult to obtain rapid progress using the classical methods of genetic improvement within breed [1-3]. ERs are nuclear receptors that share significant similarities in sequence and some of the functions. both ERa and ERb serve as transcription factors that stimulate or inhibit target genes transcription. The ovaries are the major location for estrogen production during puberty[4]. The biological actions of estrogen are mediated by binding to the ERs. Estrogen is a key hormone in chicken follicles growth with independent functions attributed to receptors[4,5]. Estrogen plays a pivotal role in the changes which occur within the follicles by stimulating the granular cells proliferation, and the facilitation of the various actions of FSH and LH on these cells [6]. There are several effects of estrogen and its receptors in addition to follicular and oviduct growth, especially when puberty approaches, as they have been shown to play role in the concentration increased of fatty acids in the blood, which helps the fat deposition in developing follicles to form oocytes, also they activate the liver metabolism to create the necessary elements to the yolk construction, especially proteins, lipids, phosphorus, vitamins, and calcium deposition in external eggshell as well as, the appearance of the secondary sexual characteristics of hen and the sexual behavior of the female [7,8]. FOXL2 is expressed in granulosa cells of growing follicles and in maturing and ovulated oocytes of hen ovaries [9,10]. So it plays an important role regulating the development of follicles and ovulation in chickens [11-15]. It has a role in increasing the number of ovarian...
follicles and their differentiation in chicken.

2. Materials and Methods

2.1. Hens and character measurements

This research was conducted at the poultry research station at Office of Agricultural Research – Ministry of Agriculture. The fieldwork continued from 1/3/2018 to 25/4/2019 (420 Days). The laboratory work began on 16/2/2019 until 16/8/2019. A total of 104 hens descend from F4th generation of brown local Iraqi chickens were used. The hens were kept at individual cages equipped with longitudinal feeders and water nipples. The dimension of each cage was 40 (length) * 40 (width) * 60 (height) cm. The preventive and hygienic programs that recommended by the Office of Agricultural Research was applied. Five types of ratios were used, according to age. The lighting program used was depended by the Office of Agricultural Research with a lighting power of 60 watts, with the lighting hours number 15 h from 15 weeks of age to the end of production period [1]. The mean of body weight, hen days egg production (HDEP) and the weight of eggs produced was calculated for haplotypes.

2.2. DNA Extraction

At 320 days of age, three ml of blood were collected from individuals of the 104 local Iraqi hens under the study via the wing vein. Genomic DNA was extracted using G- spin DNA extraction kit by intron company/ Korea. Prior DNA extraction, blood volume was modification to 10 microliters [2]. To ensure genomic DNA purity, according to [16], the agarose gel has been made in 1% condensation by melting 1 g of agarose in 100 ml of previously made TBE buffer [17]. Three µl of the processor loading dye (Intron / Korea) has been mixed with 5 µl of the supposed DNA to be electrophoresis, after the mixing process, the mixture is loaded at the gel holds. An electric current of 7 v2 has been exposed for 1.5 h till the tincture has reached to the other side of the gel. The gel has been tested by ultraviolet spectrophotometer source with 336 nm after putting the gel in the pool which contains 30µl Red safe nucleic acid staining solution and 500 ml of distilled water.

2.3. Primers designation

To find the genetic variation in ESR1, ESR2 and FOXL2 genes, primers were designed for the target gene fragment and shown in tables (1). The primers had been supplied by an integrated DNA technologies company(IDT)/Canada. Note: the FOXL2 gene primer is designed depending on the insensitive DNA strand.

| Genes | Primer | Sequence | Tm (°C) | GC (%) |
|-------|--------|----------|---------|--------|
| ESR1  | Forward| 5'-TCC AAG GTT TAG GGT CGT TCA G-3’ | 56.7 | 50 |
| ESR1  | Reverse| 5'-TTC AAC CAA AAT GGT CTC CCA GT-3’ | 56.8 | 43.5 |
| ESR2  | Forward| 5'-TCT TCC TGC TCA GCCAGT TG -3’ | 57.3 | 55 |
| ESR2  | Reverse| 5'-GCT CTG AAC TGG CCCAAG AT -3’ | 57.5 | 55 |
| FOX L2| Forward| 5'-GCC GTT GTA GGA GTT CAC CA -3’ | 57.2 | 55 |
| FOX L2| Reverse| 5'-GGG GCA AGG AGG AAC TGA G -3’ | 57.9 | 63.2 |

2.4. Polymerase chain reaction(PCR)

The total size of the reaction is 25 µl, PCR PreMix 5 µl, DNA 1.5, forward primer 1 µl, reveres primer 1µl, and DdH2O 16.5µl. The temperature has identified (Initial denaturation and annealing) after several experiments (Gradient PCR). The optimum denaturation temperatures 95°C and, annealing temperatures 64°C used to detect target regions of ESR1, and the optimum denaturation temperatures 95°C and, annealing temperatures 62°C used to detect target regions of ESR2 and FOXL2 genes.
2.5. PCR product electrophoresis

To preparation of gel, the percentage 2% of agarose was used, by melting 2 g of agarose in 100 ml of previously made TBE buffer. five μl of the PCR product was loaded into the holes of the gel and 5 μl of the ladder was loaded into the first hole of the gel. An electric current of 7 v has been exposed for 1.5h till the tincture has reached the other side of the gel for ESR1, ESR2 and 1h for FOXL2 gene as shown in figures(1,5,12) respectively. The gel has been tested by a source of the UV with 336 nm after putting the gel in the pool which contained 30µl red safe nucleic acid staining solution and 500 ml from distilled water. Note: Loading dye will not be added for the PCR product electrophoresis because the master mix contains a dye.

2.6. DNA sequencing

PCR product samples were sent to the biogen company in South Korea to determine the target regions sequence for each gene, by sequencer device, then detect the genotypes.

2.7. Sequence results analysis

For genotyping, the results of sequence were analyzed by(geneious prime program); it is the world’s leading bioinformatics software platform for molecular biology and sequence analysis. Note: an analysis of the valid samples had been adopted, which have high quality, according to the program. Note: the FOXL2 gene sequence has been transferred to the sensitive strand, then genotyping. The genotypes were determined by comparing nucleotides type and number of alleles that appeared in DNA sequence for each individual with the DNA of Gallus gallus domesticus the recorded by NCBI.

2.8. Statistical analysis

Data were statistically analyzed using the Statistical Analysis System program [18] to study the interaction relationship between the genotypes of ESR1 and FOX L2 genes with body weight and egg production traits for the F4th generation of brown local Iraqi chickens according to the mathematical model shown below and calculated the significant differences between the averages using the polynomial Duncan test.

\[
Y_{ij} = \mu + A_i + C_j + AC_{ij} + e_{ijk}
\]

Yij: the value of observation j that belongs to the genotype i.
\(\mu\): the average of the measured trait.
\(A_i\): the effect of the ESR1 gene polymorphism of the site (T57198C)
\(C_j\): the effect of the FOXL2 gene polymorphism of the site (C919T)
\(AC_{ij}\): the interaction between ESR1 and FOXL2 genes.
\(e_{ijk}\): random error which is distributed normally an average equal to zero and variance of \(\sigma^2_e\)

2.9. Polymorphism evaluations

Frequencies of genotypes and alleles at each SNP site were calculated, with each polymorphism evaluated for Hardy–Weinberg equilibrium using (Chi-square \(\chi^2\)) test to compare the percentages of genotypes for each gene (degree of freedom=1).

\[
P + q = 1
\]

\[
PA = \frac{2 \times \text{No. of Homozygous} + 1 \times \text{No. of Heterozygous}}{2 \times \text{Total number of sample}}
\]

3. Results and Discussion

3.1. PCR of estrogen hormone receptor 1 gene (ESR1)

A specific sequence of the estrogen hormone receptor 1 gene was amplified using the primers, which showed a molecular weight of 1500 bp (Figure 1).
3.2. Detection of polymorphism and genotypes in ESR1 gene

To detect polymorphism in the ESR1 gene, all samples of the PCR products were performed using the DNA sequence, as previously described. One novel polymorphism was found within the target fragment of estrogen hormone receptor1 gene, it was a single nucleotide polymorphism (SNP), T57198C transition located within ESR1 gene (intron 3), two alleles (T and C) and three genotypes were found: Wild(TT), Heterozygous(TC) and Homozygous(CC) as shown in the figures 2, 3 and 4 respectively.
Figure 2. ESR1 gene, wild genotype (TT), showed one peak, belongs to both natural alleles with nucleotide (T) marked with an arrow, which is the site of genetic variation assumed in the third intron of the ESR1, one peak for nucleotide (T) indicates to there is no genetic variation in this site of the DNA, and the birds of this group are considered wild type, with genotype TT.

Figures 3. ESR1 gene, heterozygous genotype (TC). It was visible from the figure (3) two peaks, represent two alleles, in the site of genetic variation of the third intron of the ESR1, one for the natural nucleotide (T), another for the variance nucleotide (C) where had been a transition (T > C), and marked with an arrow, and the birds of this group are considered heterozygous type, with genotype TC.

Figures 4. ESR1 gene, homozygous genotype (CC), a one peak with nucleotide (C), marked too, there had been a transition (T/ C) transition in a site of genetic variation for the third intron of the ESR1, to both alleles, were marked too, and the birds of this group are considered homozygous type, with CC genotype.

From the foregoing, there were allelic variation in the untranslated region of ESR1 gene, which is a single nucleotide polymorphism (SNP) resulting in three genotypes TT, TC and CC. The results of study agree with previous study [19], who indicated a polymorphism in untranslated regions of the ESR1 gene in blue egg-shell chicken.
3.3. Distribution of genotypes and allele frequency of ESRI gene (SNP T57198C)

There are non-significant differences between the genotypes distribution of ESRI in the F4th generation of brown local Iraqi chickens. The genotypic frequencies was 57.4%, 33.7%, and 8.9% for TT, TC and CC respectively which is followed Hardy-Weinberg equilibrium, in addition to the allele frequency of T and C was 0.743 and 0.257 respectively. The birds' numbers of different genotypes were 58, 34 and 9 for TT, TC and CC respectively (Table 2).

The assured scientific explanation that the F4th generation of brown local Iraqi chickens previously will not be sufficiently subject to an intense selection on egg production traits, so its contains a lot of polymorphism in several of DNA locus.

| The polymorphism | Genotype | No of hens | Genotype frequency | Allele | Allele frequency | $\chi^2$ |
|------------------|----------|------------|--------------------|--------|------------------|--------|
| SNP T57198C      | TT       | 58         | 57.4%              | T      | 0.743            |        |
|                  | TC       | 34         | 33.7%              | C      | 0.257            |        |
|                  | CC       | 9          | 8.9%               |        |                   | 1.44   |
|                  | Total    | 3          | 101                |        | 100%             |        |

P-value 0.49

3.4. PCR of estrogen receptor 2 gene (ESR2)

A specific sequence of the estrogen receptor 2 gene was amplified using the primers shown in the chapter of materials and methods, the size of amplified PCR product was found to be -1181 bp. Figure(5).

Figure 5. PCR amplified product of region of ESR2 on 2% agarose gel. PCR product (1181 bp), 100 bp DNA Ladder.
3.5. Detection of polymorphism and genotypes in ESR2 gene

To find genetic variation of ESR2 gene, total of samples of PCR products were analyzed using the DNA sequence, as already described. Two novel SNPs, within untranslated regions of ESR2, were assessed. Every one of polymorphism a number had been given according to its location within gene. One of the identified SNPs T39565C transition located within intron 7. Another SNP G40100A transition within third prime untranslated region (3′-UTR) was identified. The Details of DNA Sequence in the first SNP of ESR2 T39565C.

The polymorphism T39565C transition located within intron 7 with two alleles (T and C) and three genotypes were found: Wild (TT), Heterozygous (TC), and Homozygous (CC) as shown in the figures 6, 7 and 8 respectively.

![Figure 6](image1.png)

**Figure 6.** ESR2 gene, wild genotype (TT), at the location of T39565C marked of the ESR2 gene (intron 7), one peak represented by the wild allele nucleotide T was found, which indicates there is no genetic variation in this site of the DNA, this group individuals are the wild type with TT genotype.

![Figure 7](image2.png)

**Figure 7.** ESR2 gene, heterozygous genotype (TC), point out two peaks represent two alleles in the seventh intron of the ESR2 in T39565C site, for both the natural nucleotide T and the variance nucleotide C where had been a transition (T - C), group of this birds are heterozygous have TC genotype.
Figure 8. ESR2 gene, homozygous genotype (CC), it was apparent one peak with nucleotide C within ESR2 gene on T39565C site, there had been a transition (T-C) for together alleles in a site of the genetic variation of the ESR2 gene. The birds of this group were considered homozygous type, with CC genotype.

3.6. Distribution of genotypes and allele frequency for SNP T39565C of ESR2 gene

Three genotypes (TT, TC, and CC) of the ESR2 T39565C in F4th generation of brown local Iraqi chicken were noted. The percentage of genotypes 37.11%, 52.58%, and 10.31, with 36, 51 and 10 birds for each TT, TC and CC respectively. Non-significant differences showed between the percentage of these genotypes according to the Chi-square test. The allele frequency was 0.634 and 0.366 for T and C respectively, this result revealed that this population are agreement with Hardy-Weinberg equilibrium. Table (3).

Table 3. Distribution of genotypes and allele frequency for SNP T39565C of ESR2 gene.

| The polymorphism | Genotype | No of hens | Genotype frequency | Allele | Allele frequency | $\chi^2$ |
|------------------|----------|------------|-------------------|--------|-----------------|--------|
| SNP T39565C      | TT       | 36         | 37.11%            | T      | 0.634           |        |
|                  | TC       | 51         | 52.58%            | C      | 0.366           |        |
|                  | CC       | 10         | 10.31%            |        |                 | 1.7    |
| Total            | 3        | 97         | 100%              |        |                 |        |

The Details of DNA Sequence in the second SNP (G40100A) of ESR2

The polymorphism G40100A transition within third prime untranslated region (3’-UTR) with two alleles (G and A) and three genotypes were found: Wild (GG), Heterozygous (GA) and Homozygous (AA) as described in the figures 9, 10 and 11 respectively.
Figure 9. ESR2 gene, wild genotype (GG), one peak was evidenced within G40100A site, contains the wild allele nucleotide (G), and birds of this group were classified as wild type, with GG genotype.
Figure 10. ESR2 gene, heterozygous genotype (GA), a transition (G40100A) in one of birds groups was found, where two peaks appeared, represent two alleles in the site of genetic variation of ESR2 (3'-UTR), one of alleles was wild (G), another to the variance allele (A). This group is considered a heterozygous type, with GA genotype.

Figure 11. ESR2 gene, homozygous genotype (AA), mentions one peak with nucleotide (A), there had been a transition (G-A) in both alleles for a site of genetic variation (G 40100A) of the ESR2, and this group is classified homozygous type, with AA genotype.

Two polymorphism of ESRs genes are observed of untranslated regions, and it is expected there are many SNP in the non-coding regions due to the huge area these genes contain, as the molecular size of ESR1 103.705 kb, encodes only 589 amino acid and the size of ESR2 41.734 kb, protein encodes to 472 amino acid, this indicates the presence of large areas of non-coding regions of these genes that may have a lot of number of SNP as the studies mentioned that a single nucleotide polymorphisms are most common of genetic variation amongst individuals [8].

3.7. Distribution of genotypes and allele frequency for SNP G40100A of ESR2 gene

The results of table (4) indicate to three genotypes(GG, GA, and AA) were found of the ESR2 G40100A in the F4th generation of brown local Iraqi chicken. Non-significant differences in their percentages and allele frequency according to Hardy–Weinberg law. GA genotype showed the most percentage, 48.39%, next GG genotype with 43.01%, then AA Genotype 8.6%. The allele frequency of each allele G and A, is 0.672 and 0.328 respectively with the advantage of allele G.
Table 4. Distribution of genotypes and allele frequency for 3'-UTR SNP (G40100A) of ESR2 gene

| The polymorphism      | Genotype | No of hens | Genotype frequency | Allele | Allele frequency | $\chi^2$ |
|-----------------------|----------|------------|--------------------|--------|------------------|---------|
| SNP G40100A           | GG       | 40         | 43.01%             | G      | 0.672            |         |
|                       | GA       | 45         | 48.39%             | A      | 0.328            | 0.89    |
|                       | AA       | 8          | 8.6%               | _      | _                |         |
| Total                 | 3        | 93         | 100%               | _      | 1                |         |

3.8. PCR of forehead box L2 gene (FOXL2).

A specific sequence of the FOXL2 gene was amplified using the primers shown in the chapter of materials and methods, the size of amplified PCR product was found to be -625 bp, as showed in figure (12).

Figure 12. PCR amplified product of region of FOXL2 on 2% agarose gel. PCR product (625 bp), 100 bp DNA Ladder.

3.9. Detection of polymorphism and genotypes in FOXL2 gene.

To investigate polymorphism in the FOX L2 gene, all samples of the PCR products were analyzed using the DNA sequence, as formerly described. One novel polymorphism within the target fragment FOX L2 gene was identified, the (SNP), C919T transition in the gene, was located exclusively at encoding region, two alleles (C and T) and two genotypes were found: Wild(CC) and heterozygous(CT) as shown in the figures 13 and 14 respectively. The SNP C919T(FOXL2) leads to a non-synonymous substitution (threonine ACG 69- to-methionine ATG).
Figure 13. FOXL2 gene, SNP (C919T), wild genotype (CC), appeared one peak, belongs to both natural alleles with nucleotide (C) at the site of genetic variation supposed of the FOX L2 gene (Exon), that confirms there is no variation in this site of the DNA, and these birds were deemed wild type, with the genotype CC.

Figure 14. FOXL2 gene, heterozygous genotype (CT), A transition (C- T) in the second group of birds was found, where two peaks appeared, belong two alleles, in the site of polymorphism of FOXL2 gene, one for the natural allele (C), another to the differ allele (T). This group is considered a heterozygous type, with the genotype CT.

3.10. Distribution of genotypes and allele frequency for SNP C919T of FOXL2 gene

Two alleles (C and T) and two genotypes (CC and CT) of the FOXL2 C919T in the F4th generation of brown local Iraqi chicken were observed, the frequency of allele C 0.911 was higher than allele T with 0.089. This result revealed that this population are agreement with Hardy–Weinberg equilibrium. The percentage of genotype CC (82.29%) by 79 birds higher than genotype CT with percentage (17.71%) and 17 birds. Non-significant differences showed between the genotypes percentage according to the Chi-square test as shown in table (5).

The current study is similar to the results of [21] about the polymorphism in the coding region of FOXL2 gene in chicken, which leads to replace one of the amino acids, but its disagreed with them in the polymorphism site. The novel polymorphism is evidence of the diverse of mating lines using in selection programs which causes to some polymorphism emergence.

3.11. Effect of interaction the ESR1 and FOXL2 genes in body weight

It is evident from table (6). Non-significant differences between combined genotypes (Haplotypes) in the mean of body weight for ages 17 and 40 weeks despite the presence of arithmetic variance, while both body weight at age of sexual maturity and 60 weeks recorded significant differences (P<0.05) as the H4 haplotype was significantly associated with higher body weight compared to H6 haplotype (1722.8 and 1425.8g) at the age of sexual maturity and (2350 and 1900g) at 60 weeks of age for H4 and H6 respectively.

There is no previous study on the interaction between the ESR1 and FOXL2 genes, but previous studies indicated the importance of the association between these genes and other genes and their effect on body weight characteristics as well as the possibility of their use in selection programs[22,23].
Table 5. Distribution of genotypes and allele frequency for SNP C919T of FOXL2.

| The polymorphism | Genotype | No of hens | Genotype frequency | Allele | Allele frequency | $\chi^2$ |
|------------------|----------|------------|--------------------|--------|------------------|---------|
| SNP C919T        | CC       | 79         | 82.29%             | C      | 0.911            | 0.03    |
|                  | CT       | 17         | 17.71%             | T      | 0.089            |         |
| Total            | 2        | 96         | 100%               | -      | 1                | 0.8534  |

Table 6. Effect of interaction the ESR1 and FOXL2 genes in body weight.

| Body weight | Haplotype | P-Value |
|-------------|-----------|---------|
| At 17 weeks (g) | H1 = TTCC | 0.26 |
|             | H2 = TTCT |        |
|             | H3 = TCCC |        |
|             | H4 = TCCT |        |
|             | H5 = CCCC |        |
|             | H6 = CCCT |        |
| Sexual maturity (g) | H1 = TTCC | 0.05 |
|             | H2 = TTCT |        |
|             | H3 = TCCC |        |
|             | H4 = TCCT |        |
|             | H5 = CCCC |        |
|             | H6 = CCCT |        |
| At 40 weeks (g) | H1 = TTCC | 0.3 |
|             | H2 = TTCT |        |
|             | H3 = TCCC |        |
|             | H4 = TCCT |        |
|             | H5 = CCCC |        |
|             | H6 = CCCT |        |
| At 60 weeks (g) | H1 = TTCC | 0.05 |
|             | H2 = TTCT |        |
|             | H3 = TCCC |        |
|             | H4 = TCCT |        |
|             | H5 = CCCC |        |
|             | H6 = CCCT |        |

Means within a row with the different superscripts per trait are significantly different (P<0.05). *= significant at (p<0.05), N.S= non-significant.

3.12 Effect of interaction the ESR1 and FOXL2 genes in the age at sexual maturity, first egg weight, and egg-laying clutch

Table (7) showed non-significant differences between the haplotypes in the age at sexual maturity (ASM) as the rapprochement of haplotypes was observed, whereas type H5 was late mathematically in ASM (159.67, 163.63, 162.89±3.56, 167.5, 177.25 and 159.25 days) for H1, H2, H3, H4, H5, and H6 respectively, therefore this maybe cause differences in other productive traits. Also no statistically significant differences in the egg laying clutch except for the significant high (P<0.05) of the haplotype H3 compared to H4 in egg-laying clutch length (ELCL) at 30 weeks of age (4.72 and 2.46) for H3 and H4 respectively. Mathematical differences in the first egg weight for H4 compared to H5 and H6 was observed. The high standard error in the averages of the first egg weight resulted in the absence of significant differences between haplotypes. The present study agrees with [24] regard to the impact of the egg laying clutch by genetic factors, adding that egg production characters are inherited as a polygenic traits [25-27].
Table 7. Effect of interaction the ESR1 and FOXL2 genes in the age at sexual maturity, first egg weight, and egg-laying clutch.

| Traits                  | H1 (TTCC) | H2 (TTCT) | H3 (TCCC) | H4 (TCCT) | H5 (CCCC) | H6 (CCCT) | P-Value |
|-------------------------|-----------|-----------|-----------|-----------|-----------|-----------|---------|
| ASM (day)               | 159.67±2.24 | 163.63±5.23 | 162.89±3.56 | 167.5±13.27 | 177.25±18.03 | 159.25±8.2 | 0.5     |
| First egg weight (g)    | 40.01±0.68 | 38.1±1.33 | 39.61±0.85 | 42.4±2.81 | 37.62±4.25 | 37.7±2.2 | 0.56    |
| ELCN at 30 weeks        | 10.64±0.52 | 10.75±1.13 | 9.25±0.72 | 9.75±3.06 | 10.66±5.17 | 11.25±3.01 | 0.7     |
| ELCL at 30 weeks        | 4.01±0.31[^a] | 3.63±0.61[^b] | 4.72±0.41[^a] | 2.46±0.33[^b] | 2.52±0.87[^b] | 3.81±0.66[^b] | 0.05    |
| ELCN at 45 weeks        | 33.28±1.15 | 34.37±2.79 | 31.21±1.86 | 33.25±7.41 | 25.75±5.72 | 36±5.98 | 0.54    |
| ELCL at 45 weeks        | 3.6±0.21 | 3.59±0.4 | 4.07±0.31 | 3.48±0.54 | 4.36±1.21 | 3.48±0.56 | 0.73    |
| ELCN at 60 weeks        | 55.79±1.77 | 59.75±3.17 | 52.25±2.7 | 54±10.53 | 50.75±6.94 | 61±21.79 | 0.57    |
| ELCL at 60 weeks        | 2.88±1.09 | 2.96±0.2 | 3.57±0.26 | 3.38±0.54 | 3.15±0.59 | 3.11±0.41 | 0.3     |

Means within a row with the different superscripts per trait are significantly different (P<0.05). * = significant (P<0.05), N.S. = non-significant.

H1=TT(ESR1)&CC(FOXL2); H2=TT(ESR1)&CT(FOXL2); H3=TC(ESR1)&CC(FOXL2);
H4=TC(ESR1)&CT(FOXL2); H5=CC(ESR1)&CC(FOXL2); H6=CC(ESR1)&CT(FOXL2);
ASM= Age at sexual maturity; ELCN= Egg-laying clutch number; ELCL= Egg-laying clutch length

3.13. Effect of interaction the ESR1 and FOXL2 genes in the mean of egg production and egg weight

The interaction between genes resulted in many significant differences between haplotypes (Table 8). The haplotypes H3, and H4 outperformed H6 (P<0.05) in egg weight (EW) at 30 weeks (45.74, 47.01 and 41.9g) for H3, H4, and H6 respectively, while the other haplotypes did not show significant differences in EW at 30 weeks, in addition to the superiority of haplotype H4 (P<0.05) over haplotypes H2, H5, H6 in EW at 45 weeks (47.03, 52.3, 48.36and 44.82g) for H2, H4, H5, and H6 respectively, as well the outperform of haplotypes H1 and H3 (P<0.05) over the H6 haplotype in EW at 45 weeks (48.84, 48.72 and 44.82g) for H1, H3 and H6 respectively. The haplotype H4 was recorded a significant increase (P<0.05) compared to haplotypes H2 and H6 in EW at 60 weeks (48.37, 54.04 and 46.43g) for H2, H4, and H6 respectively. The haplotype H6 was characterized by a significant increase (P<0.05) in egg production at 60 weeks of age compared to the H5 haplotype (148.75 and 182.25eggs) for H5 and H6 respectively, in addition to its mathematical superiority over other haplotypes. It was observed the relationship between increase the number of eggs produced with early sexual maturity which accompanied by the decrease of egg weight for the H6 haplotype, which confirms the importance of early sexual maturity about improving egg productivity in laying hens [6].

The previous studies were pointed out the estrogen receptors role in egg production as well as egg-laying clutch in chickens [7,28]. Also other study was showed the association ESR1 gene polymorphism with egg production traits in quail [29]. The FOXL2 gene plays an important role in chickens follicles development regulating and ovulation [30-32]. A separated prior studies were showed a significant effect of the polymorphism in the FOX L2 and ESR1 genes of egg production traits, including the number of eggs produced and the eggs weight, in addition to noting the importance studying the relationship of interaction between genes and its effectiveness to increasing the variation between the haplotypes in productive traits [25,27].

So the present study support ESR1 and FOXL2 roles of hen egg production when doing a gene interaction process, which confirm the additive action for these genes to improvement the quantitative traits like egg production, egg weight and body weight, wherever the greatest yield was obtained via the study of polymorphism effect of two genes, and this maybe lead to conclude that two SNPs in ESR1 and FOXL2 genes may supply importance genetically markers of chicken breeding, via prediction and early selection for beneficial phenotypes of the body weight, and egg weight by choosing the haplotype H4 (TCCT), and the number of eggs produced by H6 (CCCT).
Table 8. Effect of interaction ESR1 and FOXL2 genes in the mean of egg production and egg weight.

| Traits                      | H1            | H2            | H3            | H4            | H5            | H6            | P-Value |
|-----------------------------|---------------|---------------|---------------|---------------|---------------|---------------|---------|
|                             | TTCC          | TTCT          | TCCC          | TCCT          | CCC           | CCCT          |         |
| HDEP at 30 weeks (No)       | 38.69±1.91    | 36.75±4.81    | 39.63±2.84    | 27.25±11.77   | 25.66±11.55   | 38±5.5        | 0.4     |
| EW at 30 weeks (g)          | 45.32±0.4ab   | 43.92±1.14ab  | 45.74±0.6a    | 47.01±1.52a   | 43.67±1.9ab   | 41.9±1.07b   | 0.05    |
| HDEP at 45 weeks (No)       | 111.5±3.38    | 115.63±5.76   | 113.78±4      | 104.5±10.26   | 94.9±9.2      | 116±2.94     | 0.54    |
| EW at 45 weeks (g)          | 48.84±0.4ab   | 47.03±1.15bc  | 48.72±0.64ab  | 52.3±2.27ab   | 48.36±2.14bc  | 44.8±0.51c   | 0.02    |
| HDEP at 60 weeks (No)       | 166.29±5.52ab | 172.13±5.78ab | 170.18±6.8ab  | 165.75±9.2b   | 148.75±10.37b | 182.25±3.68a | 0.05    |
| EW at 60 weeks (g)          | 50.29±0.48ab  | 48.37±1.3b    | 50.09±0.68ab  | 54.04±2.19a   | 50.34±1.5ab   | 46.43±0.68b  | 0.03    |

Means within a row with the different superscripts per trait are significantly different (P<0.05). *= significant (P<0.05), N.S= non-significant.
H1=TT(ESR1)&CC(FOXL2):H2=TT(ESR1)&CT(FOXL2):H3=TC(ESR1)&CC(FOXL2):
H4=TC(ESR1)&CT(FOXL2):H5=CC(ESR1)&CC(FOXL2):H6=CC(ESR1)&CT(FOXL2)
HDEP= Hen days egg production :EW= Egg weigh

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