Molecular Approaches of Candidate Genes in Genetic Improvement Programs in Poultry

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Abstract

In poultry, selection programs through quantitative genetics are time consuming in case of lowly heritable traits. Several genes that to be used in selection are regarded as candidate genes that affecting economic traits in poultry and these candidate genes have successful approaches in identifying several DNA markers associated with production and reproductive traits. Candidate genes are one of the primary methods to determine the specific genes related to the economic traits and molecular genetics techniques can be used to identify these genes or their chromosomal regions in terms of Quantitative Trait Loci (QTL) that affect these traits. Using biotechnology techniques are the best way to achieve this fast genetic improvement. This approach has enabled opportunities to enhance genetic improvement programs in poultry by direct selection on genes or genomic regions that affect economic traits through marker-assisted selection (MAS) and gene introgression. Mapping of quantitative trait loci (QTL) was the perfect approach to identify genes related to complex traits at genome-wide level. Recently, genome wide association study (**GWAS**) was used to identify the casual genes affecting economic traits in poultry using sequences variations mainly single nucleotide polymorphism (SNP). Many genes for growth and egg production were identified in poultry using **GWAS** method. The objectives of this reviewed article are: 1) To apply a fine chromosomal mapping for localizing the QTL affecting economic traits in the F_2 population using specific microsatellite markers or SNP's in chickens and possibly identify candidate genes and causative mutations associated with the economic traits, 2) Determining the molecular markers to be used for evaluating the genetic variability among poultry breeds, 3) Reporting and characterizing the candidate genes to be used in genetic improvement programs, 4) Detecting the molecular associations between candidate genes and growth, feeding and egg traits and disease resistance responses, 4) Determining the SNP genotypes and their associations with growth and feeding performances, egg traits and disease resistance responses, 5) Defining the genetic model for detecting the molecular associations between SNP genotypes of candidate gene and economic traits, 6) Performing genome wide association study (GWAS) in order to detect potential causative mutations and genomic regions affecting productive and reproductive traits in chickens, 7) Applying genomic selection program based on the estimated Genomic Breeding Values (GBV), and 8) Suggesting a genetic improvement program to improve the Egyptian chickens breeds and strains using molecular approaches.

Keywords: Poultry, Molecular applications, Candidate genes, GWAS, Genomic Breeding Values (GBV), Genomic selection.

The chromosomal QTL mapping and their positions in chicken genome:

The chicken genome consists of 38 pairs of autosomes and sex chromosomes Z and W. The chromosomes can be classified into two size groups, nine macrochromosomes and 30 microchromosomes **(Bloom *et al.*, 1993)**. Recent development of statistical methods and comprehensive linkage maps of the chicken genome has provided tools for mapping loci affecting quantitative traits **(Mackay *et al.*, 2009)**. However, only few genome-wide QTL scans have been reported in poultry, and none of these has involved egg production and egg quality traits in layers.

The identification and utilization of QTL provide the potential for more rapid genetic improvement in selection programs, especially for traits that are difficult to be improved with traditional selection **(Ikeobi *et al.*, 2002)**. In the last 15 years, several experimental chicken populations (F_0, F_1, F_2 and F_3) have been constructed from different breeds for use in gene and QTL mapping studies **(Jacobsson, 2005; Liu *et al.*, 2008; Bulut *et al.*, 2013; Abdel A'al *et al.*, 2016; 2017)**. To exemplify, the chromosomal regions affecting phenotypic traits (many growth and egg traits) in different chicken breeds have been investigated **(Tatsuda and Fujinaka 2001; Sewalem *et al.*, 2002; Li *et al.*, 2003; Sasaki *et al.*, 2004; Siwek *et al.*, 2004; Nones *et al.*, 2006; Nassar *et al.*, 2013; Abdel A'al *et al.*, 2016; 2017)**.

In Germany, **Goraga et al. (2012)** reported that: 1) A highly significant region on chromosome 4 with multiple QTL for egg production traits between 19.2 and 82.1 Mb, 2) QTL region explained 4.3 and 16.1% of the phenotypic variance for number of eggs and egg weight in the F_2 population, respectively, 3) Genome-wide suggestive QTL for egg weight were found on chromosomes 1, 5, and 9, and for number of eggs on chromosomes 5 and 7, 4) A genome-wide significant QTL affecting age at first egg was mapped on chromosome 1. **Nassar *et al.* (2013, 2015)** found that CCKAR gene had specific effects on growth trait and fat deposition in white adipose tissues using quantitative trait loci (QTL) in a population crosses between New Hampshire and White Leghorn chickens.

In Egypt, the chromosomal map to be used for detecting growth and egg traits in F_2 population are

presented in Figure 1 as cited by **Abdel A'al *et al.* (2016)**. The linkage map consists of marker loci in an order on the chromosome and the map distance between the markers. The distances are given in centiMorgan (cM, one cM is equal to one recombination event in 100 meioses) and are calculated using one of the map functions. **Abdel A'al *et al.* (2016)** reported that: A total of 19 significant genome QTL that affected body weight traits were located on seven macro-chromosomes (1, 2, 3, 4, 6, 8 and Z) and one micro-chromosome (11), 2) A total of 14 significant QTL were detected for daily gain traits, distributed over 7 distinct regions on 6 chromosomes, and their effects ranged from 2 to 8.9% of the phenotypic variation, 3) A total of 11 significant genome QTL affecting daily gain traits were located on five macro-chromosomes (1, 2, 3, 4 and 8), 4) there was statistical evidence for two QTL on chromosome 4, 5) The proportions of phenotypic variation explained by significant and suggestive QTL for body weight traits at 4, 8, 12 and 16 weeks were 21.1, 30.8, 29.3 and 25.4%, respectively.

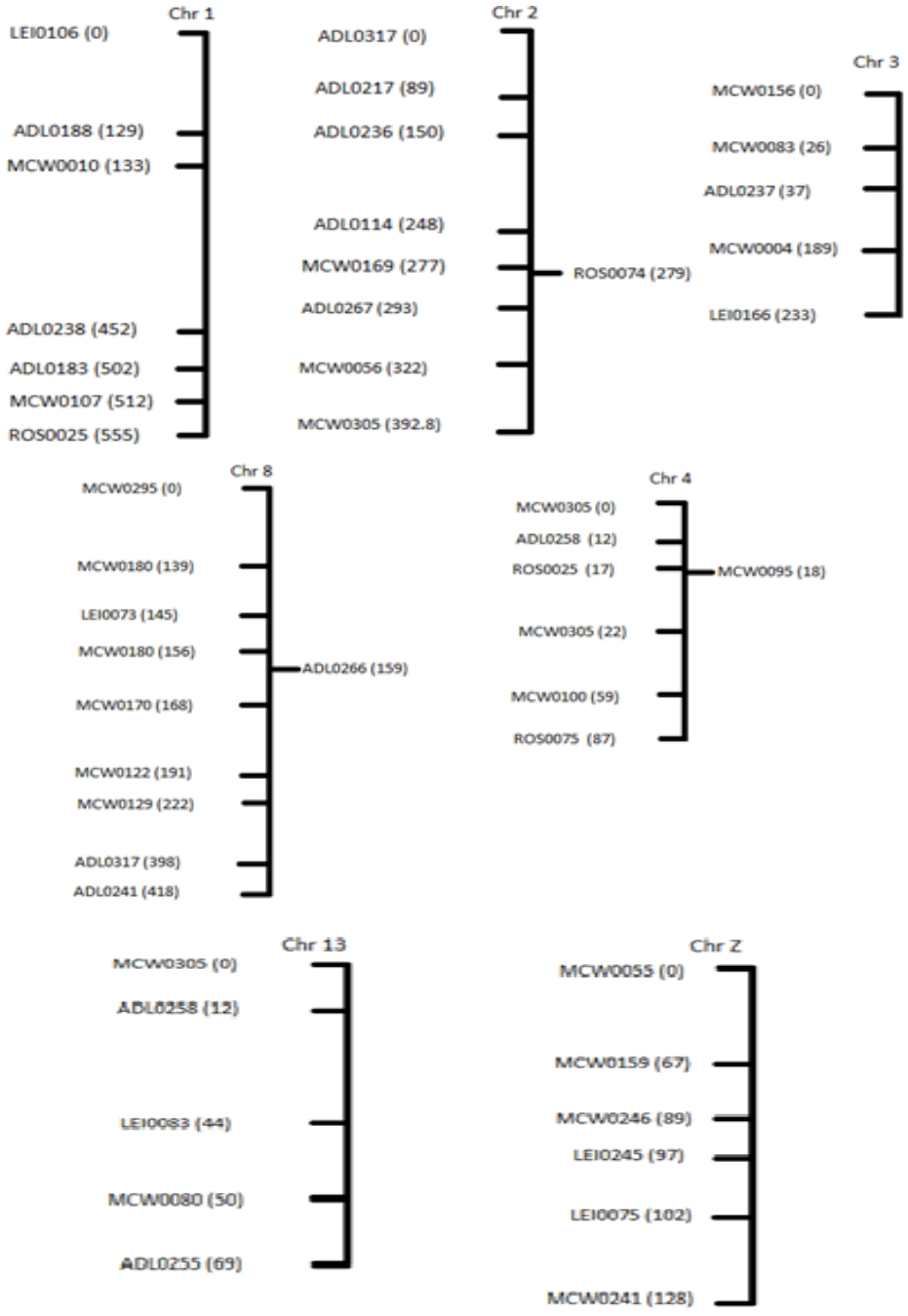

Figure 1. QTL mappings in chickens, including positions and names of the markers as cited by the Egyptian study (**Abdel A'al *et al.*, 2016)**.

The estimates on the length of the complete genome ranged from 3064 to 3800 cM, based on the mapping function and the map distances covered 48 to 60% of the whole chicken genome. The markers MCW247 (on chromosome 2) and ADL345 (on chromosome 8) have been mapped only in the Wageningen reference population, while the marker MCW170 (on chromosome 4) and markers MCW133 and ADL315 (both on chromosome 7) and marker MCW129 (on chromosome 4) have been mapped only in the East Lansing population.

For QTL mapping and their positions detected for growth traits, **Abdel A'al *et al.* (2016)** illustrated that the chromosome group, number of informative microsatellite markers, chromosome map length (*cM*), that was used for a whole genome scan of growth traits in F_2 cross are presented in Table 1. They reported that the total chromosomal map length was1901 cM ranging from 25 cM on chromosome 11 to 568 cM on chromosome 1, with an average marker spacing of 24.39 cM and that ranging from 7.8 cM on chromosome 8 to 24.3 cM on chromosome 1. The position of QTL relative to the first marker (cM) indicated that QTL were located in the region of 0 to 502 cM, 0 to 233 cM, 0 to 179 cM and 12 to 555 cM for body weights at 4, 8, 12 and 16 weeks of age, respectively. He also cited that the total chromosomal map length for egg production and egg quality traits was 1949 cM ranging from 52 cM on chromosome 11 to 542 cM on chromosome 1, with an average marker spacing of 43.3 cM and that ranging from 15.3 cM on chromosome 4 to 71.5 cM on chromosome 6.

For QTL mapping and their positions detected for egg traits, previous work has suggested that chromosome 4 may be a critical region significantly associated with the variety of egg traits **(Sewalem *et al.*, 2002; Tuiskula-Haavisto *et al.*, 2002; Sasaki *et al.*, 2004; Abdel A'al *et al.*, 2016)**.

Khalil *et al.* (2016) concluded that: 1) QTL detected for body weight on chromosomes 1, 2, 3, 4, 6, 8, 11 and Z and those detected on chromosomes 2, 3, 4, 8 and Z for egg production and egg quality traits were significant and concluded that there are different sets of genes affecting early and late body weights and egg production and egg quality traits. It is not very easy at this moment to look for candidate genes in the regions with QTL and the most important reason is that the QTL regions are still too large.

Table 1 . Chromosome (linkage) group, number of microsatellite markers and map length (cM), that was used for a whole genome scan of growth and egg traits in F_2 cross

Chromosome	Number of microsatellite markers		Chromosome map length (cM)		Average marker spacing by the chromosome (cM)	
	Growth	Egg	Growth	Egg	Growth	Egg
1	10	9	568	542	24.3	60.2
2	8	8	298	401	18.7	50.1
3	2	6	273	144	11.6	24
4	7	4	198	286	17.6	15.3
6	4	3	111	123	10.4	71.5
8	3	2	97	88	7.8	44
9	1	2	123	112	20.1	56
11	5	3	25	52	8.3	17.3
13	2	2	71	69	14.5	34.5
Z	5	6	137	132	11.5	22
Total	47	45	1901	1949	14.48	43.3

Source: Abdel A'al *et al.* (2016)

Molecular markers to be used for evaluating the genetic variability:

Molecular markers can be used to evaluate genetic variability, either within or among individuals, families, and populations. Genetic markers provide information as bioinformatics indicators about polymorphism in allelic frequency at a given locus. The availability of molecular markers in poultry allows the detailed analyses and evaluation of genetic diversity and furthermore the detection of genes influencing economically important traits. Molecular markers should not be considered as normal genes as they usually do not have any biological effect. They are identifiable DNA sequences, found in specific locations of the genome, and transmitted by inheritance from one generation to the next, allowing the assessment of genetic variability among genotypes at DNA level. In the last three decades, development of many fundamental DNA technologies such as sequencing, and PCR has helped in increasing the application of molecular markers **(Weigend, 2004)**. The majority of molecular markers used nowadays are microsatellite markers, STRs (short tandem repeats) and SNPs (single nucleated polymorphism).

Among all types of the molecular markers, the microsatellites are used as the most widely markers for the analysis of genetic diversity and population

structure in poultry **(Erhardt and Weimann (2007)**. Nowadays, DNA molecular marker techniques are widely applied in the fields of germplasm identification, phylogenetic, and genetic structural analysis **(Yang *et al.*, 2013).** Accordingly, the microsatellite has been used to develop the markers from genes and they have been referred as genic molecular markers (GMMs) or functional markers (FMs). Definite number of microsatellite markers covering nine autosomal linkage groups and the sex Z chromosome to be considered in genotyping F_0 grandparents, F_1 and F_2 offspring. These markers were selected based on the degree of polymorphism and the genome coverage recommended in the molecular genetic characterization of animal genetic resources **(FAO, 2011)**. Detailed information about selected microsatellites are available at the FAO website (www.dad.fao.org/en/refer/library/guidelin/marker.pdf). The assessment of markers was based on their positions on the consensus map. A target for marker spacing of 10 cM was used to test markers across the genome (http://www.ncbi.nlm.nih.gov/mapview and http://www.thearkdb.org).

Single Nucleotide Polymorphisms (SNPs) particularly interesting as markers was acquainted firstly by **Lander (1996)**. As stated by **Brown (1999),** SNP as a marker has the following advantages over the other types of genetic markers: 1) It has high level of polymorphism, 2) It has distribution throughout the genome, 3) It has the presence within coding regions, 4) It has introns and regions that flank genes, 5) It is simple and unambiguous assay technique, 6) It has stable Mendelian inheritance, and 7) It has low levels of spontaneous mutation, (8) SNPs are less informative due to their biallelic nature, (9) SNPs have high-resolution in whole genome allelotyping because of their abundance, even spacing, and stability across the genome, and (10) SNP technique is used to identify the paternal and maternal alleles of a given gene based on polymorphisms. Genomic selection using the SNP markers is a powerful new tool for genetic selection and this is because: 1) SNPs can be detected by a number of techniques such as PCR-RFLP, 2) SNP is relatively new technology using DNA chips that can be used for large scale screening of numerous samples in a minimal amount of time **(Fontanesi *et al.*, 2008)**, 3) SNP is the most recent contribution to study DNA sequence variation, and 4) SNP represents the most innovative molecular marker in genotyping studies. On the other hand, recent advances in high-throughput DNA sequencing, computer software and bioinformatics have been facilitated the identification of SNP. **Kumar *et al.* (2007)** examined the genetic relationships among the indigenous chicken's populations in India using 10 genotyped SNP markers of the Myostatin gene *(GDF-8)* by PCR-RFLP. Another application of SNPs in chickens was applied by **Twito *et al.* (2007)** who used 25 SNPs for different genes and chromosomes to examine the genetic relationships among 20 chicken populations using the STRUCTURE software program and they compared the SNP results with the analysis using microsatellites and concluded that: 1) Microsatellites provide high clustering success due to high polymorphic nature, 2) SNPs provide broader genome coverage and reliable estimates of genetic relatedness in the genome, and 3) SNP considered to be an efficient and cost-effective genetic tool.

Molecular characterization of candidate genes in poultry breeds:

The candidate genes in local poultry breeds must be characterized in terms of the following genetic parameters:

1) Allelic and genotypic frequencies to be calculated.
2) The genetic diversity of candidate genes to be assessed by calculating the effective number of alleles *(Ne*), the observed (*Ho*) and expected (*He*) heterozygosity using GENALEX software, version 6.5 **(Peakall and Smouse, 2012)**.
3) Hardy-Weinberg equilibrium (HWE) within each population to be estimated using GENEPOP program **(Raymond, 1995);** http://genepop.curtin.edu.au/**)** performing the Chi-square test for each genetic group studied.
4) The polymorphism information content (PIC) to be calculated using CERVUS software, version 3 **(Kalinowski *et al.*, 2007)**:
5) The F-statistics of the reduction in heterozygosity due to inbreeding within each population (F_{IS}) to be calculated using GENEPOP software, version 3.4 **(Raymond, 1995);** http://genepop.curtin.edu.au/).

Molecular associations between candidate genes and growth and feeding traits:

The associations' studies cited in Table 2 have been investigated to clarify the relationship between candidate genes and growth and feeding traits in poultry. The concept of these associations could be summarized as follows:

1) Genes located on chromosome 1: The Pituitary specific transcription factor-1 (Pit-1) ***(*Jin *et al.*, 2018)**, pituitary-specific positive transcription factor 1 **(Nie *et al.*, 2008)**, Thyroid hormone responsive spot 14 α **(Cao *et al.*, 2007)**, Interferon-γ *(IFN-γ)* **(Zhou *et al.*, 2001; Ye *et al.*, 2006; Ahmed, 2010**, Inhibitor of apoptosis protein-1 *(IAP1)* **(Liu and Lamont, 2003; Ye *et al.*, 2006)**, Insulin-like growth factor *(IGF1)* **(Amills *et al.*, 2003; Wei *et al.*, 2009)**,
2) Genes located on chromosome 2: Insulin-like growth factor (IGF2) **(Amills *et al.*, 2003)**, Insulin-like growth factor binding protein *(IGFBP)* 1 and 3 ***(Ye et al.*, 2006; *Ou et al.*, 2009; Zhao *et al.*, 2015)**, Accessory protein

of the toll like receptor 4 *(MD-2)* **(Ye *et al.*, 2006),**

3) Genes located on chromosome 3: Ornithine decarboxylase *(ODC)* **(Ye *et al.*, 2006; Uemoto *et al.*, 2011; Cahyadi *et al.*, 2013)**, Gallinacins 2 to 5 (*Gal 2 to Gal 5*) **(Saleh, 2019) ,**
4) Genes located on chromosome 4: Cholecystokinin type A receptor *(CCKAR)* **(Rikimaru *et al.*, 2012; Yi *et al.*, 2018)**, Interleukin-2 *(IL-2)* **(Kramer *et al.*, 2003; Ye *et al.*, 2006; Kazemi *et al.*, 2018)**, Tumor necrosis factor-related apoptosis-inducing ligand *(TRAIL)* **(Ye *et al.*, 2006)**, Bone morphogenetic protein receptor 1B (BMPR-1B) **(Niknafs *et al.*, 2012; Ashraf and El-Tarabany, 2015)**,
5) Genes located on chromosome 5: Calpain 3 **(Zhang *et al.*, 2009)**, Transforming growth factor-β3 *(TGF-β3)* **(Ye *et al.*, 2006)**, Insulin *(INS)* **(Lei *et al.*, 2007)**,
6) Genes located on chromosome 7: Insulin-like growth factor binding protein-2 *(IGFBP-2)* **(Lei *et al.*, 2005; Leng *et al.*, 2009)**,
7) Genes located on chromosome 8: Leptin receptor gene *(LEPR)* **(Park *et al.*, 2006; El Moujahid *et al.*, 2014)**,
8) Genes located on chromosome 9: Growth hormone secretagogue receptor *(GHSR)* **(Fang *et al.*, 2010),**
9) Genes located on chromosome 10: Insulin-like growth factor 1 receptor **(Lei *et al.*, 2008)**,
10) Genes located on chromosome 15: Macrophage migration inhibitory factor *(MIF)* **(Malek *et al.*, 2004; Ye *et al.*, 2006)**,
11) Genes located on chromosome 17: Toll-like receptor 4 *(TLR4)* **(Malek *et al.*, 2004)**,
12) Genes located on chromosome 19: Inducible nitric oxide synthase *(iNOS)* **(Kramer *et al.*, 2003; Ye *et al.*, 2006)**; Caspase-1 *(CASP1)* **(Liu and Lamont, 2003; Zhou and Lamont, 2003a; Ye *et al.*, 2006)**,
13) Genes located on chromosome 20: Bone morphogenetic protein-7 *(BMP7)* **(Ye *et al.*, 2006)** located on chromosome 21: PR domain containing 16 *(PRDM16)* **(Cahyadi *et al.*, 2013)**,
14) Genes located on chromosome 26: Thyroid-stimulating hormone beta subunit *(TSH-β)* **(Lei *et al.*, 2007; Seo *et al.*, 2013)**,
15) Genes located on chromosome 27: chicken growth hormones *(GH)* and *(GH1)* **(Nie *et al.*, 2005; Anh *et al.*, 2015),**
16) Genes located on chromosome Z: Growth hormone receptor *(GHR)* **(Lei *et al.*, 2007)**.

Table 2. Candidate genes associated with growth and feeding traits in poultry as cited in literature

Chr*	Candidate gene	Trait	Breed	Reference
1	Pituitary-specific transcription factor-1 **(*Pit-1*)**	Body weight, daily gain, feed intake, feed conversion.	White Recessive Rock × Xinghua Chinese (F_2), Yellow meat type N202 and N301	**Nie *et al.* (2008)** **Jin *et al.* (2018)**
1	Thyroid hormone responsive spot 14 α	Body weight, daily gain.	Broiler × Local Chinese (F_2)	**Cao *et al.* (2007)**
1	Interferon-γ **(*IFN-γ*)**	Body weight, feed conversion.	Fayoumi ×White Leghorn (F_2) Commercial broiler	**Ahmed (2010)** **Ye *et al.* (2006)**
1	Inhibitor of apoptosis protein-1 **(*IAP1*)**	Body weight, daily gain, feed intake.	Fayoumi and 2 MHC-congenic Leghorn lines (G-B1 and G-B2) Commercial broiler	**Liu and Lamont, (2003)** **Ye *et al.* (2006)**
1	Chicken-B-cell marker **(*CHB6*)**	Body weight, feed conversion.	Commercial broiler	**Ye *et al.* (2006)**
1	Insulin-like growth factor ***(IGF1)***	Body weight, daily gain, feed intake.	Black Penedesenca (PN and MN)	**Amills *et al.* (2003); Wei *et al.* (2009)**
2	Insulin-like growth factor ***(IGF2)***	Body weight, daily gain, feed intake.	Black Penedesenca (PN and MN)	**Amills *et al.* (2003)**
2	Insulin-like growth factor binding, protein ***(IGFBP)*** 1 and 3	Body weight, feed conversion.	Commercial broiler Jinghai Yellow	**Ye *et al.* (2006)** **Ou *et al.* (2009)** **Zhao *et al.* (2015)**
2	Accessory protein of the toll like receptor 4 ***(MD-2)***	Body weight, feed conversion.	Commercial broiler	**Ye et al. (2006)**
3	Ornithine decarboxylase ***(ODC)***	Body weight, feed conversion.	Commercial broiler Korean native chicken Korean native chicken (Black: 90, Grey-Brown: 110 ،Red-	**Ye *et al.* (2006)** **Uemoto *et al.* (2011)** **Cahyadi *et al.* (2013)**

			Brown: 134, White: 125, and Yellow-Brown: 131)	
3	Gallinacins 2 to 5 ***(Gal 2 to Gal 5)***	Body weight, daily gain.	Fayomi and Rhode Island Red and their crosses	**Saleh (2019)**
4	Cholecystokinin type A receptor ***(CCKAR)***	Body weight, feed conversion.	Hinai-dori breed Tianlu Black N416	**Rikimaru *et al.* (2012)** **Yi *et al.* (2018)**
4	Interleukin-2 (***IL-2)***	Body weight, feed conversion.	Commercial broiler Mazandaran native	**Ye *et al.* (2006)** **Kazemi *et al.* (2018)**
4	Tumor necrosis factor-related apoptosis-inducing ligand (***TRAIL)***	Body weight, feed conversion.	Commercial broiler	**Ye *et al.* (2006)**
4	Bone morphogenetic protein receptor 1B **(BMPR-1B)**	Body weight.	Mazandaran native Fayoumi, Rhode Island Red	**Niknafs *et al.* (2012)** **Ashraf and El-Tarabany (2015)**
5	Calpain 3	Body weight, daily gain.	Commercial lines (S01, S02, S03, S05, and D99)	**Zhang *et al.* (2009)**
5	Transforming growth factor-β3 (***TGF-β3)***	Body weight, feed conversion.	Commercial broiler	**Ye *et al.* (2006)**
5	Insulin ***(INS)***	Body weight, feed conversion.	Xinghua × White Recessive Rock	**Qiu *et al.* (2006); Lei *et al.* (2007)**
7	Insulin-like growth factor binding protein-2 ***(IGFBP-2)***	Body weight, daily gain.	White Recessive Rock × Xinghua	**Lei *et al.* (2005); Leng *et al.* (2009)**
8	Leptin receptor gene (***LEPR***)	Body weight,feed intake, feed conversion.	Yellow meat type N202 and N301	**El Moujahid *et al.* (2014)**
9	Growth hormone secretagogue receptor **(GHSR)**	Body weight, daily gain.	White Recessive Rock × Xinghua	**Fang *et al.* (2010)**
10	Insulin-like growth factor 1 receptor	Body weight, daily gain.	XH, Taihe Silkie, Bei-jing Fatty, Yangshan, Dwarf, White Leghorn, and White Recessive Rock	**Lei *et al.* (2008)**
15	Macrophage migration inhibitory factor (***MIF)***	Body weight, feed conversion.	Commercial broiler	**Ye *et al.* (2006)**
16	major histocompatibility complex ***MHC Class II***	Body weight.	Commercial broiler Leung hang khao	**Ye *et al.* (2006)** **Molee *et al.* (2016)**
17	Toll-like receptor 4 (***TLR4***)	Body weight, feed conversion.	Korean Native Black, Rhode Island Red, Cornish	**Lim *et al.* (2013)**
19	Inducible nitric oxide synthase (***INOS***)	Body weight, feed conversion.	Commercial broiler Korean Native Black, Rhode Island Red, Cornish	**Ye *et al.* (2006);** **Lim *et al.* (2013)**
19	Caspase-1 (***CASP1)***	Body weight, feed conversion.	Fayoumi and 2 MHC-congenic Leghorn lines (G-B1 and G-B2) Commercial broiler	**Liu and Lamont (2003)** **Ye *et al.* (2006)**
20	Bone morphogenetic protein-7 (***BMP7***)	Body weight, feed conversion.	Commercial broiler	**Ye *et al.* (2006)**
21	PR domain containing 16 ***(PRDM16)***	Body weight.	Korean native chicken	**Cahyadi *et al.* (2013)**
26	Thyroid-stimulating hormone beta subunit (***TSH-β***)	Body weight.	Xinghua × White Recessive Rock Korean Native Black, Rhode Island Red, Cornish	**Lei *et al.* (2007)** **Seo *et al.* (2013)**

27	Growth hormone **(GH)** and ***(GH1)***	Body weight, daily gain.	Xinghua × White Recessive Rock	**Nie *et al.* (2005)**
			PS broiler ×Thai synthetic breeds; Kaen Thong (KT), Khai Mook Esarn (KM), Soi Nin (SN), and Soi Pet (SP)	**Anh *et al.* (2015)**
Z	Growth hormone receptor **(GHR)**	Body weight, daily gain.	White Recessive Rock × Xinghua Chinese chicken	**Lei *et al.* (2007)**

*Chr = chromosome number.

Results of **Zhou *et al.* (2005)** found significant associations (*P<0.05*) between *IGF1*-SNP and average daily gains in the F_2 generation of hybrids (Leghorn×broiler and Fayoumi×broiler). Also, the *IGF-1* gene polymorphism is associated with growth in chickens as reported by others **(Seo *et al.*, 2001; Kita *et al.*, 2005; Li *et al.*, 2009)**. **Nie *et al.* (2005)** found that SNP maintained significant associations with almost all growth traits, in F_2 cross (White Recessive Rock Xinghua Chinese chicken). **Ahmed (2010)** found that body weight at 12 wks of age was associated with *IFNG* SNP in Fayoumi chickens. **Gouda and Essawy (2010)** analyzed the polymorphism of *IGF-I* gene among Egyptian chicken breeds and indicated their significant effects on growth traits of chicken. **Ashraf and El-Tarabany (2015)** found that the A287G SNP of *BMPR-1B* gene was associated significantly with body weight at 2nd (*P*=0.02), 3rd (*P*=0.03), 4th (*P*=0.01), 5th (*P*=0.03), 6th (*P*=0.001), 7th (*P*=0.008) and 8th (*P*=0.01) week of age. **Ouyang *et al.* (2016)** indicated that two SNP of the *GNPDA2* gene were significantly associated with body weight and a number of fatness traits in chicken (P <0.05). **Lim *et al.* (2013)** found that the SNP of iNOS gene had a significant association with body weight at 270 days of age (p<0.05) in both Korean Native Black and Rhode Island Red whereas SNP of *TLR 4* gene showed insignificant association with body weight (p>0.05). **Zhao *et al.* (2015)** detected the association between *IGFBP-2* and body weight in Jinghai Yellow chickens and three reference chicken populations (Arbor Acre, Youxi, and Bian chickens). **Molee *et al.* (2016)** identified seven SNPs of Major Histocompatibility Complex class II gene (C125T, A126T, C209G, C242T, A243T, C244T and A254T) and stated that significant associations between all SNPs and body weight. **Kazemi *et al.* (2018)** reported significant associations between the *IL-2* gene polymorphisms at promoter region and body weight at 8 weeks of age in Mazandaran native fowls (P≤0.05). **El Moujahid *et al.* (2014)** reported that four SNPs of leptin receptor gene were significantly associated with body weight at 49 and 70 day of age (*P* < 0.05), and feed intake (*P* < 0.05) in the yellow N202 strain, and feed conversion (*P* <0.01) in the yellow N301 strain. **Yi *et al.* (2018)** found that C334A SNP of Cholecystokinin type A receptor *(CCKAR)* gene was associated with feed intake (P < 0.01) and significantly associated with the daily gain (P < 0.05) in Chinese local chicken Ianlu Black pure-line N416. **Jin *et al.* (2018)** found that SNP of *Pit-1* gene was associated significantly with feed intake (p<0.05), and body weight at 70 days of age and feed conversion (p<0.05).

Molecular associations between candidate genes and egg traits:

Many researchers have studied the associations between candidate genes and egg production and egg quality traits in poultry **(Cui *et al.*, 2006; Li *et al.*, 2009; Xu *et al.*, 2011a; 2011b; Zhu and Jiang, 2014; Ngu *et al.*, 2015; Vu and Ngu (2016); Charoensook *et al.*, 2016; Osman *et al.*, 2017; Nguyen *et al.*, 2018; Azmal *et al.*, 2019; Bhattacharya *et al.*, 2019).** These molecular associations' studies cited in Table 2 could be outlined as follows:

1) Genes located on chromosome 1: Insulin -like Growth Factor I *(IGF-I)* **(Li *et al.*, 2009; Ngu *et al.*, 2015)**, Melatonin Receptor 1B *(MTNR1B)* **(Li *et al.*, 2013)**, Matrix metallopeptidase 13 **(Yuan *et al.*, 2016),**
2) Genes located on chromosome 2: Neuropeptide Y *(NPY)* **(Xu *et al.*, 2011b; Nguyen *et al.*, 2015)**, Gonadotropin releasing hormone I *(GnRHI)* (**Bhattacharya *et al.*, 2019)**, Prolactin *(PRL)* **(Cui *et al.*, 2006; Kulibaba 2015; Osman *et al.*, 2017; Nguyen *et al.*, 2018),** Vasoactive intestinal peptide receptor- 1 *(VIPR1)* (**Xu *et al.*, 2011b; Nguyen *et al.*, 2018),**
3) Genes located on chromosome 3: Vasoactive intestinal peptide *(VIP)* (**Zhou *et al.*, 2010; Nguyen *et al.*, 2018),** Follicle-stimulating hormone receptor *(FSHR)* (**Li *et al.*, 2011),**
4) Genes located on chromosome 4: Melatonin Receptor 1A and 1C *(MTNR1A)* and *(MTNR1C)* (**Li *et al.*, 2013)**, Gonadotropin releasing hormone II *(GnRHII)* **(Bhattacharya *et al.*, 2019),**
5) Genes located on chromosome 5: gremlin *(GREM1)* and *(GREM2)* ***(Tyasi et al., 2018),***
6) Genes located on chromosome 7: Inhibin α *(INHA)* **(Cui et al., 2019),**
7) Genes located on chromosome 9: Ovocalyxin-32 **(Fulton *et al.*, 2012),**

8) Genes located on chromosome 13: Growth Differentiation Factor 9 Gene (GDF9) **(Liu *et al.*, 2018)**, Dopamine receptor D1 *(DRD1)* **(Tempfli *et al.*, 2015),** Rap guanine nucleotide exchange factor 6 *(RAPGEF6)* **(Azmal *et al.*, 2019),**
9) Genes located on chromosome 20: Matrix metalloproteinases *(MMP9)* (**Zhu and Jiang, 2014)**,
10) Genes located on chromosome 24: Dopamine D2 Receptor *(DRD2)* (**Xu *et al.*, 2011a; 2011b; Ngu *et al.*, 2015),**
11) Genes located on chromosome 27: Growth hormone *(GH)* **(Su *et al.*, 2014; Kulibaba, 2015; Vu and Ngu 2016),** Single transducers and activators of transcriptions 5B *(STAT5B)* (**Charoensook *et al.*, 2016)**
12) Genes located on chromosome Z: Prolactin receptor gene *(PRLR)* (**Kulibaba, 2015),** growth hormone receptor (**Kulibaba, 2015).**

Table 3. Candidate genes associated with egg production and egg quality traits in poultry as cited in literature

Chr*	Candidate gene	Trait	Breed	Reference
1	Insulin -like Growth Factor I *(IGF-I)*	Egg production	Erlang Mountain Chicken	**Li *et al.* (2009)**
			Noi Chickens	**Ngu *et al.* (2015)**
1	Melatonin Receptor 1B *(MTNR1B)*	Egg production	Erlang Mountain Chicken	**Li *et al.* (2013)**
1	Matrix metallopeptidase 13	Egg production	Hy-line Brown	**Yuan *et al.* (2016)**
2	Neuropeptide Y *(NPY)*	Egg production	Ningdu Sanhuang chickens	**Xu *et al.* (2011b)**
			Noi Chickens	**Ngu *et al.* (2015)**
2	Gonadotropin Releasing Hormone I *(GnRHI)*	Egg production Egg quality	White Leghorn	**Bhattacharya *et al.* (2019)**
2	Prolactin (*PRL)*	Egg production	Lien Minh chicken, Poltava clay chicken, Hubbard F15, Lohman, Cobb500, Avian48	**Cui *et al.* (2006); Kulibaba (2015); Osman *et al.* (2017)**
			Lien Minh chicken	**Nguyen *et al.* (2018)**
2	Vasoactive Intestinal Peptide Receptor- 1 *(VIPR1)*	Egg production	Ningdu Sanhuang chickens	**Xu *et al.* (2011b)**
			Lien Minh chicken	**Nguyen *et al.* (2018)**
3	Vasoactive Intestinal Peptide (*VIP)*	Egg production	Ningdu Sanhuang chickens	**Zhou *et al.* 2010)**
			Lien Minh chicken	**Nguyen *et al.* (2018)**
3	Follicle Stimulating Hormone Receptor *(FSHR)*	Egg production	(Zang, Jining Bairi, Wenchang, Luqin, Xinyang Brown, Lohmann Brown chickens)	**Li *et al.* (2011)**
4	Melatonin Receptor 1A and 1C *(MTNR1A)* and *(MTNR1C)*	Egg production	Erlang Mountain Chicken	**Li *et al.* (2013)**
4	Gonadotropin Releasing Hormone II *(GnRHII)*	Egg production Egg quality	White Leghorn	**Bhattacharya *et al.* (2019)**
5	Gremlin *(GREM1)* and *(GREM2)*	Egg production	Chinese Dagu chickens	**Tyasi *et al.* (2018)**
7	Inhibin α (INHA)	Egg production	Lu Hua chicken	**Cui *et al.* (2019)**
9	Ovocalyxin-32	Egg production Egg quality	White Leghorn, White Plymouth Rock, Rhode Island Red	**Fulton *et al.* (2012)**
13	Growth Differentiation Factor 9 Gene *(GDF9)*	Egg production	Lu Hua chicken	**Liu *et al.* (2018)**

13	Dopamine receptor D1 *(DRD1)*	Egg production	Hungarian Yellow	**Tempfli *et al.* (2015)**
13	Rap Guanine Nucleotide Exchange Factor 6 *(RAPGEF6)*	Egg production	Chinese Jing Hong	**Azmal *et al.* (2019)**
20	Matrix Metalloproteinases *(MMP9)*	Egg production	Xinyang brown, Hy-line brown, Wenchang	**Zhu and Jiang (2014)**
24	Dopamine D2 Receptor *(DRD2)*	Egg production	Ningdu Sanhuang chickens, Lien Minh chicken	**Xu *et al.* (2011a; 2011b)**
			Noi Chickens	**Ngu *et al.* (2015)**
27	Gowth Hormone *(GH)*	Egg production	Recessive White chicken, Qingyuanpatridge chickens,	**Su *et al.* (2014)**
			Poltava clay chicken	**Kulibaba (2015)**
			Noi Chickens	**Vu and Ngu (2016)**
27	Single Transducers and Activators of Transcriptions 5B *(STAT5B)*	Egg production Egg quality	White Leghorn, Rhode Island Red	**Charoensook *et al.* (2016)**
Z	Prolactin Receptor *(PRLR)*	Egg production	Poltava clay chicken	**Kulibaba (2015)**
Z	Growth Hormone Receptor	Egg production	Poltava clay chicken	**Kulibaba (2015)**

*Chr = chromosome number.

Molecular associations between candidate genes and immune traits:

In the last two decades, several studies have reported the associations of immune genes with immune response, bacterial burden and antibody titers against Salmonella in chickens **(Zhou *et al.*, 2001; Lamont *et al.*, 2002; Kramer *et al.*, 2003; Liu and Lamont, 2003; Malek and Lamont, 2003; Zhou and Lamont, 2003a; Malek *et al.*, 2004; Ahmed, 2010; Cahyadi *et al.*, 2013; Khatab *et al.*, 2017; Saleh, 2019).** The research conducted on candidate genes associated with immunity traits could be summarized in Table 4. The concept of these molecular associations in terms of chromosome number could be illustrated as follows:

1) Genes located on chromosome 1: Dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 1A *(DYRK1A)* **(Ghebremicael *et al.*, 2008)**, and Inhibitor of Apoptosis Protein-1 *(IAP1)* **(Kaiser and Lamont, 2002; Tohidi *et al.*, 2013)**, Cluster of Differentiation 28 *(CD28)* **(Malek *et al.*, 2004)**, Interferon-γ *(IFN-γ)* **(Sadeyen *et al.*, 2006; Kazemi *et al.*, 2018)**,
2) Genes located on chromosome 2: Myeloid differentiation primary response gene 88 (MYD88) **(Liu *et al.*, 2015)**, Accessory protein of the toll like receptor 4 (MD-2),
3) Genes located on chromosome 3: Transforming growth factor β2 *(TGF-β2)* and β4 *(TGF-β4)* **(Kramer *et al.*, 2003; Tohidi *et al.*, 2013; Muhsinin *et al.*, 2017)**, Gallinacins 1 to 13 (*Gal 1 to Gal 13*) (**Hasenstein *et al.*, 2006 and Hasenstein and Lamont, 2007, Saleh, 2019)**,
4) Genes located on chromosome 4: TNF-related apoptosis-inducing; ligand (TRAIL); Interleukin 2 (IL2) and 8 (IL8) **(Kramer *et al.*, 2003; Kazemi *et al.*, 2018)**,
5) Genes located on chromosome 5: Tumor necrosisfactor-related apoptosis-inducing ligand *(TRAIL*) **(Mariani *et al.*, 2001; Tohidi *et al.*, 2013)**, Transforming growth factor β3 *(TGFβ3)* **(Kramer *et al.*, 2003; Tohidi *et al.*, 2013; Psifidi *et al.*, 2016)**,
6) Genes located on chromosome 6: Prosaposin *(PSAP)* **(Kramer *et al.*, 2003)**,
7) Genes located on chromosome 7: Natural resistance-associated, protein 1 *(NRAMP1)* **(Kramer *et al.*, 2003; Tohidi *et al.*, 2013; Psifidi *et al.*, 2016)**,
8) Genes located on chromosome 14: Lipopolysaccharide- induced tumor necrosis factor *(TNF)* α factor *(LITAF)* **(Malek *et al.*, 2004; Tohidi *et al.*, 2013)**,
9) Genes located on chromosome 15: Macrophage migration inhibitory factor (MIF) **(Malek *et al.*, 2004)**, immunoglobulin lambda-like polypeptide 1 *(IgL)* **(Kramer *et al.*, 2003)**, (10) Genes located on chromosome 16: Major histocompatibility complex MHC Class I and II, Major histocompatibility complex MHC class I α1, α2 and β1 domain **(Zhou and Lamont, 2003b)**,

10) Genes located on chromosome 17: Toll like receptor 4 *(TLR4)* **(Hu *et al.*, 1997; Malek *et al.*, 2004; Khatab *et al.*, 2017)**,
11) Genes located on chromosome 19: Caspase 1 *(CASP1)* **(Kramer *et al.*, 2003)**, Inducible nitr c oxide synthase *(iNOS)* **(Kramer *et al.*, 2003)**,
12) Genes located on chromosome 24: Interleukin 18 *(IL-18)* **(Sadeyen *et al.*, 2006; Kazemi *et al.*, 2018)**,
13) Genes located on chromosome 26: Polymeric immunoglobulin receptor *(PIGR)*, Map kinase-activated protein kinase 2 *(MAPKAPK2)*, Interleukin 10 *(IL10)* and Ligatin *(LGTN)* **(Ghebremicael *et al.*, 2008)**.

Table 4. Genes associated with immunity traits in chickens as cited in literature

Chr*	Candidate gene	Trait	Breed or line	Reference
1	Dual Specificity tyrosine-(Y) Phosphorylation Regulated Kinase1A (***DYRK1A***)	Bacterial load	Leghorn Fayoumi	**Ghebremicael *et al.* (2008)**
1	Cluster of Differentiation 28 ***(CD28)***	Bacterial load, antibody response to Salmonella enteritidis vaccine	Fayoumi and 2 MHC-congenic Leghorn lines (G-B1 and G-B2)	**Malek *et al.* (2004)**
1	Inhibitor of Apoptosis Protein-1 ***(IAP1)***	Bacterial load	Fayoumi and 2 MHC-congenic Leghorn lines (G-B1 and G-B2)	**Kaiser and Lamont (2002)**
			Fayoumi and 2 MHC-congenic Leghorn lines (G-B1 and G-B2)	**Liu and Lamont (2003)**
			Village, Red Junglefowl	**Tohidi *et al.* (2013)**
1	Interferon-γ **(*IFN-γ*)**	Bacterial load	Inbred lines 61 and 15I	**Sadeyen *et al.* (2006)**
			Mazandaran native	**Kazemi *et al.* (2018)**
2	Myeloid Differentiation Primary Response 88 ***(MYD88)***	Bacterial load	Laying hen	**Liu *et al.* (2015)**
2	Accessory protein of the toll like receptor 4 ***(MD-2)***	Bacterial load	Fayoumi and 2 MHC-congenic Leghorn lines (G-B1 and G-B2)	**Malek *et al.* (2004)**
3	Transforming growth factor β4 ***(TGF-β4)***	Bacterial load	Old Dutch breeds, Broilers	**Kramer *et al.* (2003)**
			Village, Red Junglefowl	**Tohidi *et al.* (2013)**
			Sentul chickens	**Psifidi *et al.* (2016)**
3	Transforming growth factor β2 ***(TGF-β2)***	Bacterial load, antibody response to *Salmonella enteritidis* vaccine	Old Dutch Broilers	**Kramer *et al*. (2003)**
			Sentul chicken	**Muhsinin *et al.* (2017)**
3	Gallinacins 1 to 13 ***(Gal 1 to Gal 13)***	Bacterial load, antibody response to *Salmonella enteritidis* vaccine	Broiler× Fayoumi	**Hasenstein *et al.* (2006); Hasenstein and Lamont (2007)**
			Fayomi and Rhode Island Red and their crosses	**Saleh (2019)**
4	TNF-related apoptosis-inducing ligand **(*TRAIL*)**	Bacterial load	Fayoumi and two MHC-congenic Leghorn lines (G-B1 and G-B2)	**Malek and Lamont (2003)**

			Village, Red Jungle fowl	**Tohidi *et al.* (2013)**
4	Interleukin 2 ***(IL2)***	Bacterial load	Old Dutch breeds, Broilers	**Kramer *et al.* (2003)**
4	Interleukin 8 ***(IL8)***	Bacterial load	Old Dutch breeds, Broilers	**Kramer *et al.* (2003)**
			Mazandaran native	**Kazemi *et al.* (2018)**
5	Tumor necrosis factor-related apoptosis-inducing ligand (***TRAIL)***	Bacterial load (*Salmonella typhimurium*)	Village, Red Jungle fowl	**Tohidi *et al. (2013)***
5	Transforming growth factor β3 ***(TGFβ3)***	Bacterial load	Old Dutch breeds, Broilers	**Kramer *et al.* (2003)**
			Village Red Jungle fowl	**Tohidi *et al.* (2013)**
			Sentul chickens	**Psifidi *et al.* (2016)**
6	Prosaposin ***(PSAP)***	Bacterial load	Old Dutch breeds, Broilers	**Kramer *et al.* (2003)**
			Fayoumi and 2 MHC-congenic Leghorn lines (G-B1 and G-B2)	**Lamont *et al.*, 2002**
7	Natural resistance-associated protein 1 ***(NRAMP1)***	Bacterial load	Fayoumi and 2 MHC-congenic Leghorn lines (G-B1 and G-B2)	**Lamont *et al.*, 2002**
			Laying hen	**Liu *et al.* (2015)**
			Sentul chickens	**Psifidi *et al.* (2016)**
14	Lipopolysaccharide-induced tumor necrosis factor (TNF) α factor **(*LITAF*)**	Bacterial load, antibody response to *Salmonella enteritidis* vaccine	Fayoumi and 2 MHC-congenic Leghorn lines (G-B1 and G-B2)	**Malek *et al.* (2004)**
			Village, Red Jungle fowl	**Tohidi *et al.* (2013)**
15	Macrophage migration inhibitory factor (***MIF)***	Bacterial load, antibody response to *Salmonella enteritidis* vaccine	Fayoumi and 2 MHC-congenic Leghorn lines (G-B1 and G-B2)	**Malek *et al.* (2004)**
15	Immunoglobulin lambda-like polypeptide 1 ***(IgL)***	Bacterial load Antibody response to *Salmonella enteritidis* vaccine	Old Dutch breeds, Broilers	**Kramer *et al.* (2003)**
16	Major Histocompatibility Complex ***MHC class I α1,α2, β1 domain***	Antibody response to *Salmonella enteritidis* vaccine	MHC-congenic Fayoumi × G-B1 Leghorn	**Zhou and Lamont (2003b)**
17	formin binding protein 1 ***(ADL0293)***	Antibody response to *Salmonella enteritidis* vaccine	Low antibody line × High antibody line (F_1 and F_2)	**Yunis *et al.* (2002)**
17	Toll like receptor 4 ***(TLR4)***	Bacterial load and Antibody response to *Salmonella enteritidis* vaccine	Fayoumi and 2 MHC-congenic Leghorn lines (G-B1 and G-B2)	**Malek *et al.* (2004)**
			Fayoumi, Hyline	**Khatab *et al.* (2017)**
19	Caspase 1 ***(CASP1)***	Bacterial load	Old Dutch breeds, Broilers	**Kramer *et al.* (2003)**
			Village Red Junglefowl	**Tohidi *et al.* (2013)**
19	Inducible nitric oxide	Bacterial load	Old Dutch breeds,	**Kramer *et al.* (2003)**

	synthase **(*iNOS*)**		Broilers	
24	Interleukin ***18 (IL-18)***	Bacterial load	Inbred lines 61 and 15I	**Sadeyen *et al.* (2006)**
			Mazandaran native	**Kazemi *et al.* (2018)**
26	Polymeric immunoglobulin receptor ***(PIGR)***	Bacterial load	Leghorn × Fayoumi	**Ghebremicael *et al.* (2008)**
26	Map kinase activated protein kinase 2 (***MAPKAPK2***)	Bacterial load	Leghorn × Fayoumi	**Ghebremicael *et al.* (2008)**
26	Interleukin 10 ***(IL10)***	Bacterial load	Leghorn × Fayoumi	**Ghebremicael *et al.* (2008)**
26	Ligatin ***(LGTN)***	Bacterial load	Leghorn × Fayoumi	**Ghebremicael *et al.* (2008)**
16	Major histocompatibility complex ***MHC Class I***	Bacterial load (*Salmonella enteritidis*)	Fayoumi and 2 MHC-congenic Leghorn lines (G-B1 and G-B2)	**Lamont *et al. (*2002)**
			MHC-congenic Fayoumi × G-B1 Leghorn	**Zhou and Lamont (2003b)**

*Chr = chromosome number.

Results of **Ghebremicael *et al.* (2008)** showed that: 1) The *MAPKAPK2* and *IL10* genes were highly associated with Salmonella enteritidis burden in spleen tissue and cecal luminal content (P < 0.001), 2) The associations of *PIGR* with spleen tissue and cecal content were suggestive (P < 0.05), and 3) SNP in *MAPKAPK2* and *IL10* were strongly associated with Salmonella burden and may be valuable in generating resistant birds by marker-assisted selection. **Malek *et al.* (2004)** stated that the *CD28* broiler sire SNP was associated with both bacterial load in the cecum (P < 0.003) and vaccine antibody response (P < 0.05), while the *MD2* SNP was associated (P < 0.04) with the bacterial load in the spleen. In Malaysia, **Tohidi *et al.* (2013)** summarized their results in that: 1) The *NRAMP1-SacI* polymorphism correlated with Salmonella Enteritidis load in the cecum (P = 0.002) and spleen (P = 0.01) of Village chickens, 2) The polymorphisms in the restriction enzyme of *TGFβ3-BsrI, TGFβ4-MboII,* and *TRAIL-StyI* were associated with Salmonella Enteritidis burden in the cecum, spleen, and liver of Village chickens and Red Jungle fowl (P < 0.05), and 3) The *NRAMP1*, *TGFβ3*, *TGFβ4*, and *TRAIL* genes are potential candidates genes to be used in selection programs for increasing genetic resistance against *Salmonella Enteritidis* in native Malaysian chickens.

In the last two decades, some studies have been performed to detect the gene structure of Salmonella and its ability to resist against antibiotics **(Dehkordi *et al.,* 2015).** Susceptibility to *Salmonella Pullorum* infection suggesting that *MyD88* gene may be one of the major *Salmonella Pullorum* resistant genes in innate immune system **(Liu *et al.,* 2015)**. The use of SNP of innate immune genes, such as natural resistance associated macrophage protein 1 Nramp1 **(Beaumont *et al.,* 2003)**, *CD28* and *MD2* **(Malek *et al.,* 2004)** and *TLR4* **(Li *et al.,* 2010)** lead to enhancement of *Salmonella Pullorum* resistance in chicken. The expression of *TLR4* and immune related genes, such as *Gal 1*, *Gal 2*, *IL-8*, *IL-18*, and *IFN-ɣ*, established different degrees of correlation against salmonella in hens **(Sadeyen *et al.,* 2006)**. **Ahmed (2010)** demonstrated that the novel *IFNG* promoter SNP was associated with antibody kinetics for *Brucella abortus* (BA) and Sheep Red Blood Cells (SRBC) in laying hens, suggesting that this cytokine may play a pivotal role in the relationship between immune function and growth.

SNP genotypes and their associations with growth and feeding performance:

Harini *et al.* (2013) reported that the TC genotype had heavier body weight than chickswith genotype TT at all ages. Nevertheless, **Anh *et al.* (2015)** reported that genotypes of AG and GG having similar positive effects on chicken growth**. Zhao *et al.* (2015)** reported that chickens of the AA genotype of *IGFBP-2* gene had significantly heavier body weight at hatch and 12 weeks of age, than those of the AB genotype ($p<0.05$), while there were no significant differences among different genotypes in body weight at 4, 8 and 16 weeks of age ($p>0.05$). **Seo *et al.* (2013)** found a significant association between TSH- β SNP and body weight at day 150 in Cornish chickens where chicks of CC genotype (302 ± 6.3 g) were significantly heavier than that of the GG genotype (294 ± 4.5 g) ($p<0.05$). **Jin *et al.* (2018)** found that chicks with the TT genotype of SNP of *Pit-1* gene had heavier and significant body weight at 70 day than those of the CT and CC genotypes and feed conversion was just the opposite ($p<0.05$), while the AA genotype had heavier and

significant body weight at 70 day and lower feed conversion than those of AT and TT genotypes.

SNP genotypes and their associations with egg production and egg quality traits:

Li *et al.* (2013) found that two SNPs genotypes of *MTNR1A* and *MTNR1C* genes were significantly associated (P< 0.01) with egg number at 300 days of age (EN) and age at first egg (AFE). **Kulibaba (2015)** stated that chicks with heterozygous genotype AB of *GH* gene in Poltavskaya Glinistaya chickens were characterized by higher egg productivity than chicks with genotype BB. **Vu and Ngu (2016)** also found that genotypes of *GH* gene were associated with egg production in Noi Chickens. **Xu *et al.* (2011a)** found that SNP of *VIPR-1* gene associated significantly (P<0.05) with AFE. **Xu *et al.* (2011b)** reported that SNPs genotypes of *VIPR-1* were associated significantly (P<0.001) with EN300 and total egg production and chicks of genotype CC had lower total egg production after 300 days compared to TT genotype in Ningdu Sanhuang laying chickens. **Ngu *et al.* (2015)** reported significant associations between genotypes and egg numbers (P<0.05) at 28-47 weeks of age in Noi chicken for *IPR-1/TaqI* and for *VIPR-1/HhaI)* of Vietnam. **Tempfli *et al.* (2015)** found that the genotypes of *PRL* gene were associated significantly with egg production (*P*<0.05), whereas genotypes of *DRD1* gene associated significantly with egg production at 45 weeks of age (*P*<0.05). **Charoensook *et al.* (2016)** showed that the genotypes of *STAT5B* gene were significantly associated with egg weight, egg height, shell weight, shell thickness, albumen weight and yolk color (p<0.001) and chicks of genotype GG had better egg quality traits than AA and AG genotypes. **Liu *et al.* (2018)** found that SNP of growth differentiation factor 9 gene was significantly associated with AFE (0.04) and WFE (0.03) and the CC genotype exhibited higher AFE and WFE values than those of TT genotype. **Azmal *et al.* (2019)** showed that genotypes of RAPGEF6 gene were significantly associated (p < 0.0001) with egg-laying rate at 60 days in Chinese local Jing Hong layer chickens.

SNP genotypes and their associations with disease resistance responses:

Muhsinin *et al.* (2016) reported that the CC genotype was significantly lower in *salmonella pullorum* count than TC and TT genotypes of *NMAMP1* gene in Sentul chickens (p<0.05). **Khatab *et al.* (2017)** reported that Fayoumi breed is a pure Egyptian conserved breed with one genotype (BB) for *TLR4*-exon 2 in resistant and susceptible birds compared with Hy-line strain chickens, which have different genotypes (AB and BB), where BB genotype frequency was higher in susceptible birds. So, *EcoRI-TLR4*-exon 2 could not use as a marker for susceptibility or resistance to salmonella infection in Fayoumi breed, i.e. it may be used in Hy-line strain for detection the sensitive birds through BB genotype and *Taq1-TLR4*-exon 1 can be used as a marker for selection resistant birds to salmonella infection in Hy-line strain through AB genotype. **Saleh (2019)** found that the SNP genotypes of *GAL 3*, *GAL 4* and *GAL* 5 genes were significantly associated with the caecal *Salmonella typhimurium* count and the antibodies produced (p<0.05) in Fayoumi (F), Rhode Island Red (R), ½R½F and ½F½R genetic groups.

Model for detecting the molecular associations between SNP genotypes of candidate gene and economic traits:

To detect the molecular associations between the genotypes of candidate gene and economic traits, the effects of SNP genotype on different traits must be estimated using the PEST software **(Groeneveld, 2006)** and applying the following multi-trait animal model (defined in matrix notation):

$$\mathbf{y= Xb + Z_a u_a + e}$$

Where **y** = vector of observed trait on the bird; **b** = vector of fixed effects, like sex, genetic group, genotype of candidate gene (three genotypes); **X** and $\mathbf{Z_a}$ = incidence matrices corresponding to the fixed and additive random effects of the bird ($\mathbf{u_a}$), respectively; **e** = vector of random residual effects.

Genome-wide association studies (GWAS) with economic traits in chickens:

Recently, with advances in technologies of next generation sequencing, genome-wide association studies (GWAS) have been used successfully to identify single nucleotide polymorphisms (SNPs) and candidate genes associated with quantitative traits. GWAS have revealed important regions associated with production, reproduction and disease resistance traits in chickens **(Yuan *et al.*, 2015; Fan *et al.*, 2017)**. One of the essential elements in GWAS is a powerful statistical method that can be employed to identify genetic associations. Methods that using model of population structure by estimating the covariance due to genetic correlation between individuals have been reported to perform better in terms of detecting true associations than models that ignore genomic relationship matrix **(Gianola *et al.*, 2016)**.

In the last decade, a remarkable range of discoveries from genome-wide association studies (GWASs) have been detected in chickens **(Moser *et al.*, 2009; Xu *et al.*, 2013; Sun *et al.*, 2015; Yuan *et al.*, 2015; Gianola *et al.*, 2016; Psifidi *et al.*, 2016; Fan *et al.*, 2017; Pértille *et al.*, 2017; Azmal *et al.*, 2019; Kudinov *et al.*, 2019; Liu *et al.*, 2019; Qu *et al.*, 2019)**. GWAS results have been shown to be useful for selection of phenotypic traits by a customized gene chip. **Xu *et al.* (2013)** reported that chromosome 1 and 4 are the two critical

chromosomes influencing growth traits particularly body weight in chickens. **Pértille *et al.* (2017)** observed that twenty significant SNPs (P < 7.86E-07) were associated with feed conversion at 35 days and one significant SNP associated with body weight at 35 days of age, while 92 suggestive (P < 1.57E-05) SNPs were associated with feed conversion, feed intake, feed efficiency, birth weight, and body weight at 35 and 41 days of age. **Yi *et al.* (2015)** and **Qu *et al.* (2019)** identified candidate genes on GGA4 that provide strong confirmation of their previous reported region for egg weight **(Yi *et al.*, 2015)** and egg shell traits **(Sun *et al.*, 2015)**. GWAS results of **Azmal *et al.* (2019)** showed that five identified SNPs in chromosome 13 were associated with egg production traits of 120 laying birds, which are all located in RAPGEF6 gene. **Liu *et al.* (2019)** found that seven univariate GWAS for age at first egg and egg numbers were performed independently, from which a total of 161 candidate SNPs located on GGA1, GGA2, GGA5, GGA6, GGA9 and GGA24 were identified, thirteen SNP located on GGA6 that may associated with AFE and *PRLHR* gene that may affect AFE through regulating oxytocin secretion in chickens and sixteen genome-wide significant SNPs associated with EN3. **Liu *et al.* (2019)** found that genes POLA1, PDK3, PRDX4 and APOO identified by annotating sixteen genome-wide significant SNPs that can be considered as candidates associated with EN3 and did not find any candidate gene for the total egg number. **Kudinov *et al.* (2019)** observed significant associations for yield of extraembryonic fluid, age at first egg, body weight and egg weight in genotyping of 146 birds in GWASs and reported that on chromosome 2, there was an association with immune resistance in the white day old chick down colour (DOCDC).

Suggested genetic improvement program in the Egyptian chickens using molecular approaches:

Using traditional selection for genetic improvement in poultry will cause slow and low genetic progress and using biotechnology techniques are the best way to achieve fast genetic improvement. The necessary steps to perform a genetic improvement program in the Egyptian breeds of chickens using the molecular applications could be summarized as follows:

Step No	Procedure and Executable Approach
1.	**Recording the phenotypic data from full pedigree file for all birds (hens and cocks) to evaluate the birds genetically:** Adequate number of birds will be used. Not adequate records must be discarded to ensure a homogenous data set. Pedigreed birds will be used to estimate the breeding value for the economic traits in local breeds of chickens. The breeding value (EBV) will be estimated by an animal model using BLUPF90 software **(Misztal et al., 2018)** fitting univariate approach. The assumed model will be: $$\mathbf{y = Xb + Za + e}$$ where, y= vector of observations, b= vector of fixed effects with an incidence matrix X, a= vector of random bird effects with incidence matrix Z, and e= vector of random residual effects
2.	**Determine the list of main equipments required and the main list of chemicals for DNA extraction:** The necessary equipments chemicals are: PCR machine, Real-time PCR, Gel electrophoresis, Gel Documentation System, Vortex, Centrifuge 30000 rpm under cooling, Biosafety cabinet, EDTA, Ethidium Bromide, Magnesium chloride, dNTPs, PCR Master Mix (2X), Sybr green master mix kits, PFU Taq DNA Polymerase, Agarose, Phenol (nucleic acid grade), DNA isolation Kit from animal tissues, Micropipettes set, Eppendorf.
3.	**Collecting the blood samples and DNA extraction (Abdel A'al *et al.*, 2016; 2017):** The blood samples will be collected under sterile conditions by jugular vein puncture using 5-ml vacuum tubes of polypropylene containing EDTA. The samples will transfer to the laboratory in iceboxes containing ice packs and stored at -20° C until extract the genomic DNA. Genomic DNA extraction: genomic DNA will extract using a standard phenol-chloroform extraction protocol and ethanol precipitation methods**.**
4.	**Reporting candidate genes from QTLs data base:** For bovine genome, a list of previously reported QTL for economic traits was obtained from animal QTL db, release 30 **(Hu *et al.*, 2016)** (http://www.animalgenome.org/QTLdb).
5.	**Preparing the genotyping files (Liu *et al.*, 2019) and genotyping the birds:** The birds will be genotyped using SNPs markers.
6.	**Applying the Genome-Wide Association Study (GWAS):** The birds with more than 20% missing marker genotype will excluded from the analysis. A SNP will be removed from the analysis if it had minor allele frequency less than 0.02. Filtration of the marker data was performed with Plink software **(Purcell *et al.*, 2007).** A genome wide association study will performed using linear regression model in the way of regressing the average daily deviations on SNP

alleles and will be implemented by Plink software. The PLINK software will be used for analyzing the GWAS using the following model:

$$y = xb+e$$

Where, y is a vector of each GBVs of the genotyped individuals, x is each SNP information and b is coefficient value for x vector.

7. **Applying SNP association test:**
The National Center for Biotechnology Information (NCBI) database will be used to detect the genes closely associated with economic traits in poultry.

8. **Estimating the genomic breeding values (GBV or GCTA) to be applied in genomic selection:**
The genomic breeding values (GBV) will be estimated as the sum of the effects of dense genetic markers, or haplotypes of these markers, across the entire genome capturing all the quantitative trait loci (QTL) that contribute to variation in a trait. The QTL effects detected from individual single nucleotide polymorphism (SNP) markers, are first estimated in a large reference population with phenotypic information **(Abdel A'al *et al.*, 2016; 2017)**. In subsequent generations or in related populations, only marker information is required to calculate GBV.

9. **Evaluating the prediction accuracy (EBV vs GBV) :**
The correlation between the estimated traditional breeding values (EBV; using phenotypic data and pedigree) and the genomic breeding values (GBV) must be estimated. The reliability of GBV and the correlation between EBV and GBV were used to evaluate the prediction accuracy **(Moser *et al.*, 2009).**

10. **Estimating the Genomic Best Linear Unbiased Predictions (GBLUP) and SNP-GBLUP:**
The mixed model will be used to estimate the breeding values GBLUP and best linear unbiased estimation. These models estimate the fixed effects such as sex and SNPs and the random effects for a given quantitative phenotype. The proposed mixed model and its solution are presented as follows:

$$y = Xb+Zu+e$$

Where y is the vector of phenotypic values, X and Z are the design matrices; b and u are vectors of fixed and random effects, respectively. To compare the estimated breeding values (EBV) of the total SNPs with trimmed SNPs, we will use the G-BLUP which adopts the genomic relationship matrix (GRM) with total pruned SNPs and SNP-GBLUP which utilizes the SNP-SNP relationship matrix with trimmed SNPs **(Lee et al., 2014).**

11. **Applying genomic selection program (GS):**
The genomic selection (GS) is a form of marker assisted selection in which genetic markers covering the whole genome are used so that all quantitative trait loci (QTL). This approach has become feasible due to revolution in SNP discovery method like gene sequencing and SNP genotyping on DNA chip.
The genomic breeding values (GBV) and their reliabilities for the genotyped birds will be used to select the best cocks and hens based on their GBV to be parents for the next generation (genomic selection).

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