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Using of molecular genetics techniques in poultry breeding: Subject review

Ismail Y. AL-Hadeedy*[1], Sarmad T. Abdulazeez[1], Mohammed A. Mohammed[1], Imad M. Al-Jabari[2]
[1]Animal production department, college of agriculture, Kirkuk university, Kirkuk, Iraq.
[2] Nursing department, technical college of health and medical, sulaimani polytechnic university, Sulaimania, Iraq.
* E-mail: ismail.younis@uokirkuk.edu.iq

Abstract: Adopting modern molecular biology techniques to improve bird productivity by focusing on the behavior of the target gene has become a more efficient and more important method in poultry breeding programs, as it is difficult to achieve rapid progress using traditional methods of genetic breeding that depend on the phenotype, which expresses to the genotype carried by the bird. And then selecting the best birds based on phenotype and making those birds parents of next generation by mating them. This process takes a long time, arduous effort and high economic cost, until the molecular genetics techniques used that depend on discover the genes carried by the bird in cell nucleus and thus early prediction of productivity of birds without need for field breeding or depends on phenotype and thus reduce effort, time and the cost of breeding that takes several generations. The revolution in the using molecular genetics techniques began in the twentieth century, specifically in 1966, when the genetic locus of quantitative traits was revealed that are considered more important in farm animals, and what resulted then in extraction of DNA, which contributed to identifying the chromosomal locus that affect the traits, followed by the ability to multiplied DNA by using PCR techniques for the first time in 1983. Then RFLP technology was discovered, which developed DNA maps resulting from genetic variation by using restriction enzymes, this technology resulted in creation of famous chicken breeding commercial company Hy-Line International. Then came the third-generation techniques of molecular genetics known as SNP techniques, which were discovered in 1990 and considered important because it is suitable for showing phenotypic diversity that cannot be reached by other types of markers. From the foregoing, it appears that there is a revolution upcoming in the field of using modern molecular genetics technologies as a means of selection in poultry, and for those reasons this subject review was done.

Key words: molecular genetics, selection, DNA extraction, PCR, RFLP, SNP.

Introduction:

Egg production is a vital process that is regulated by the pituitary gland through a set of complex genes that control egg production (Abdel Amir et al., 2019). Because it is difficult to achieve rapid progress using traditional methods of genetic improvement of egg production (Al-khatib et al., 2016). Modern molecular biology techniques have been adopted to improve egg production by focusing on the behavior of the target gene. These techniques are a more efficient way to characterize egg production in chickens (Qin et al., 2015). It is becoming increasingly important in poultry breeding programs (Zhang et al., 2012). The basic and important step in molecular studies is to identify the genes responsible for the studied traits through the use of polymorphisms of candidate genes to assist in the selection of economically important traits. Molecular genetics techniques also provide good opportunities for selecting animals with early ages and a wide range of productive traits (Dekkers, 2004). Therefore, the most important challenges that researchers face with genetic improvement are identifying the genes that are related to the phenotypic traits and thus determining the polymorphism of the gene (Polymorphism) responsible for those traits in order to predict the effect of genotypes on the external appearance of the traits, so relying on molecular genetics will be better. From relying on the external appearance (Phenotype), as all genetic information is available at early ages, which allows early selection and thus reducing all expenses (Dickerson, 2004). The application of advances in the candidate gene approach to large-scale identification of the relationship between important genes and traits (Kulibaba and Podstreshnyi, 2012), as researchers are constantly searching for potential genes that influence the productivity and quality of traits in poultry, this approach is promising. Due the

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determination of genotypes at now can be done quickly and at a low cost due to the great development in molecular genetics techniques, especially in genetic improvement programs for farm animals and poultry (Dekkers and Hospital, 2002). Therefore, selection based on genetic parameters offers several benefits including rapid detection, accuracy, improved productivity and increased adaptability to the animals' environment (Liu, 2007).

Use of molecular genetics in genetic improvement of poultry:

Molecular genetics is the science that deals with studying the components of the cell at the molecular level, tracing its pathways, determining its functions and the vital processes it performs (Fulton, 2008). It is also concerned with how genes are duplicated, the genetic system maintained, and how mutations occur. It is also concerned with the molecular changes of nucleic acids and how they are reconstituted and their parts exchanged (Boichard et al., 2012) and work on mapping genes and genetic forms that have a role in productive traits and the possibility of adoption of these structures in genetic improvement and breeding (Naqvi, 2007). Despite the position of molecular genetics among other biological sciences, it is a science of recent origin. In the 1960s, the genetic locations of quantitative traits were revealed and the use of molecular genetics to study genetic asymmetry in animal populations (Lewontin and Hubby, 1966). Whereas, an estimate of the association between genetic loci for quantitative traits known as QTL (Quantitative Trait Loci) was revealed in 1970 (Jayakar, 1970). In 1980, genetic locations for quantitative traits and chromosomal mapping were determined in agricultural animals using nucleotide sequencing techniques (Hammer et al., 1985), while selection in agricultural animals began using genetic markers in 1990 (Cambell et al., 1996). Genetic markers are genes that are simply inherited and serve as a marker for genes or other genetic loci that control the genetic loci of quantitative traits (QTL). Therefore, it is possible to identify the genes for quantitative traits on the gene loci for the quantitative trait individually by knowing the marker allele with a known effect associated with this loci and depends on the distance between them, and this is done through crossing between lines or breeds and that these Indicators are not necessarily genes with a function, but they can be a sequence of nucleotides that can be traced in the genetic content of the animal, and it has been discovered that some nucleotide sequences repeat in genotype without having a specific known function (Weller, 2001 and Khalil, 2011). During the last decade of the twentieth century was the beginning of the use of computers to discover and locate quantitative traits QTLs affects to economic traits of large numbers of agricultural animals (Kinghorn et al., 1993; Mott, 2000; Mott and Flint, 2002). In past, reliance was on comparing differences between animals through the external appearance (Phenotype) and physiology (Physiology) that affects the trait under study but using molecular techniques, relies on a comparative study that includes direct and indirect information for DNA sequences and identifying genes or the chromosomal locus that affect to the trait (Al-Zuhairi, 2013). Accordingly, Rasouli et al. (2013) indicated that traditional selection methods were not sufficiently effective in improving the required traits in poultry because most of these traits were not genetically evaluated accurately, but with the development of molecular techniques, the studied gene became the means to improve the rate of genetic progression of the studied trait. In this regard, Stella (2008) explained that relying on molecular genetics led to obtaining more accurate information than if relying on traditional methods of selection such as adopting the external appearance, as applications of molecular genetics give information at young ages and thus lead to a reduction in costs for the breeder in order to Access to the ages at which selection takes place and the possibility of providing information about the trait to be selected. Relying on molecular information led to the discovery of the characteristics and structures of the required trait and thus working to select and improve them to be parents for subsequent generations without relying on traditional methods that require time, effort and high cost such as offspring tests (Hassan, 2011). Reliance on molecular genetics leads to the improvement of important and economic traits in birds, such as egg production, because diagnosing genes that determine the expression of economically important traits is an important research focus in genomics, as these traits have the advantage of genetic variation in the expression of those genes and in locations called Quantitative Trait Loci (QTL). Therefore, it is possible to rely on the identification and characterization of chromosomal Loci that carry the QTL on Marker Assisted Selection (MAS) programs. Molecular association maps, in combination with statistical methods, facilitated the genetic characterization of complex traits, and poultry birds are a suitable model example for this role because of its short life cycle and

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abundant production of offspring, especially quail, due to its economic importance as a good producer of meat and eggs without any food additives (Bahmanimehr, 2012).

Gene Expression:

Gene expression refers to the ability of genes to build structural and functional proteins (enzymes, hormones, etc.). This process takes place in two stages: transcription and translation (Brueckner et al., 2009). Hartwell et al. (2010) defined gene expression as the process of producing an effective protein through Working on transforming the informational storage in the gene, and to complete this process, two important stages must be passed: transcription and translation, as well as some processes regulating the structure of the producing protein (figure 1)

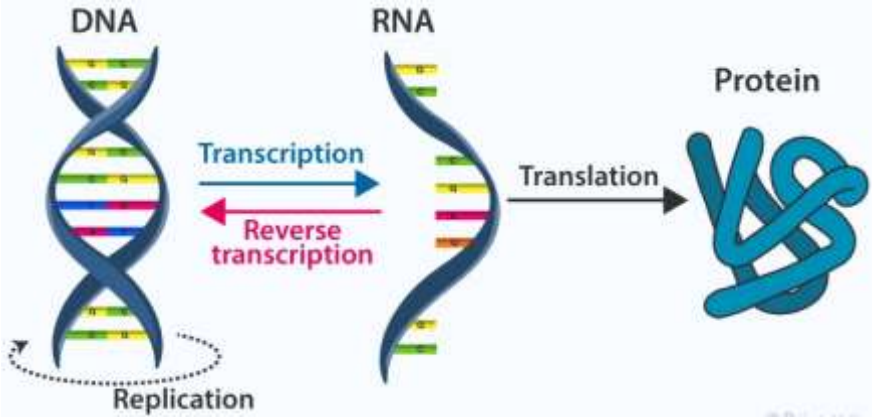

Figure 1: Gene expression of DNA

Blows and McGuigan (2016) indicated that gene expression products are often proteins that use information from (mRNA) to manufacture them, and there are non-protein products such as rRNA, tRNA, and snRNA. The result of this produces is a functional RNA. Gene expression is essential for maintaining cellular homeostasis and through it, the cell becomes able to adapt to changing environmental stresses and meet changing cell requirements (D'souza et al., 2018). As Sirri et al. (2008) showed, gene expression controls cell structure and functions and is the basis for cell differentiation in terms of morphology, diversity, and adaptability, and has a profound impact on the functions of genes in cell. Cell phenotype is also controlled by gene expression and cellular synthesis related to growth, differentiation, and survival are general reflections in changing patterns of gene expression (Wooten and Quaranta, 2017). At the genetic level, Hutt (1949) indicated that the gene that is responsible for the emergence of a trait cannot show its effect unless the environmental conditions suitable for its work are available and the cell has the ability to adapt to changing environmental pressures and meet their requirements through mitochondrial gene expression, which is the basis for maintaining their homeostasis and regulating the synthesis and degradation of RNA, and thus the stability of mitochondrial-encoded proteins (D'souza et al., 2018). In line with what was mentioned previously, it must be noted that the process of regulating gene expression takes place in a number of steps along the path of genetic information, the first of these steps is the regulation of gene expression at the genome level. At this step, the gene structure is modified, which affects to identity of the available sequences to occurrence of cloning process or the rate at which these sequences are cloned, the second step of regulating gene expression is regulation at the cloning level. In this step, the cell senses any increase in cellular protein production above the specified limit in order to save energy, and this sensitization is implemented through cloning starting point, The third step to regulate gene expression occurs at the post-transcriptional level. In this step, the mRNA molecule changes dramatically before its translation process takes place, where the hood is added at the 5' end, and a polyadenine is added to the 3' end. This mutation affects to the rate of translation and the amino acid content of the protein. Therefore, RNA stability plays a major role in regulating gene expression. The fourth step of regulating gene expression occurs at the level of translation, which is a complex process that requires a large number of enzymes, protein factors, and RNA molecules, as well as the abundance of amino acids and the nature of mRNA sequences. All these factors affect the rate of proteins produced and the regulation of gene

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expression, and the fifth and final step of the process of regulating gene expression occurs at the post-translational level, and then many proteins mutate after their synthesis, and these modifications affect the activity of proteins, and thus genes can be regulated through processes that effect on Post-translation changes, Thus, the gene is affected by regulatory activities at these steps (Nestler and Hyman, 2002; Al-Shehaib et al., 2013; Shreya, 2023).

DNA Extraction:

The genetic material contains information about all the characteristics of an organism in its DNA and is packaged in the form of chromosomes (Alberts et al., 2002). DNA can be found in nuclear chromosomes and organelles such as mitochondria and chloroplasts (Timmis et al., 2004). To study DNA for certain purposes, special techniques are needed to perform DNA extraction. Laboratory procedures for extracting DNA from a tissue or organ include disrupting cell walls and nuclear membranes, denaturing nucleoproteins, inactivating nucleases, and then isolating DNA from other cellular components (Tan and Yiap, 2009). DNA isolation is a process that removes molecules from the cell that are not needed to obtain pure DNA. The DNA sequence in every cell of a eukaryotic tissue or organ is identical with the exception of gametocytes and B lymphocytes. Thus, genomic DNA represents all the genetic information of an organism. The object can be obtained from any organ containing the cell (Simmons, 2008). Samples for extraction of animal or human DNA can be obtained from blood, semen, epithelial cells from the mouth and mucous membranes, hair, and even bones and teeth (Butler, 2012). The selection of materials or samples for DNA isolation should take into account the stress levels of the laboratory animals so that they are not damaged, and the isolation method should also be appropriate to the type of animal. such as short-term requirements or large amounts of DNA. There are several methods of DNA extraction from animal cells that are generally divided into conventional extraction and solid phase extraction (Tan and Yiap, 2009). Conventional extraction uses liquid phase principles such as the phenol chloroform-isoamyl alcohol (PCI) lysis buffer method, the salt separation method, and the chelex method. The chelex method is widely used for forensic purposes because the process is fast but does not produce pure DNA (Butler, 2012). Solid phase extraction based on the solid phase principle in the column by binding the nucleic acid in the extraction process depending on the pH value and the buffer salt content. Silica matrix, nitrocellulose, nylon, diatomaceous earth and magnetic beads can be used for the solid phases. The advantage of solid phase extraction is a fast and efficient process as it is not disturbed by incomplete liquid phase separations and is non-toxic (Tan and Yiap, 2009). Feathers are another epidermal structure made up of keratin that forms the outer covering in birds. Feather growth begins with the growth of the follicle (embryo), the pin feathers, so the brood feathers are complete (Prum and Brush, 2002). The function of feathers is to insulate the body from heat and cold from the outside environment, to help warm the body and to help with flight. DNA isolation in chickens is usually done using blood samples. In chicks, they often have small blood vessels that make it difficult to draw blood, so feathers can be used as a material for genomic DNA isolation, especially if sample numbers d' birds are reared or remain in chicks. young. Therefore, this study aimed to compare the concentration and purity of DNA isolated from lysis buffer-phenol chloroform isoamyl alcohol (PCI) and chelex method.

Polymerase chain Reaction (PCR):

The DNA chain reaction was first described by Kary Mullis in 1983 on the basis that the action of this technology is to replicate a specific piece of DNA produced from the whole genome enzymatically and in vivo with the presence of primers that bind to its complement sequence. This process is a simulation of what happens in nature in all living organisms, whose genetic material doubles during division. The interaction is simple and its contents exist since the beginning of life before Mullis implements it outside the living body and he reached this by chance when he was searching for a method to diagnose the genetic mutation that causes sick cell disease, so he found a way to duplicate a piece of DNA that is present in very small quantities to conduct the necessary studies on it (Pavlov et al., 2004). The importance of the polymerase chain reaction (PCR) is due to its many advantages, such as accuracy, specificity, and high sensitivity in detecting a specific piece of DNA among thousands of pieces. Therefore, it has become indispensable in studies of molecular genetics, in addition to being a relatively easy, fast, and private way of working when needs to analyze many samples. Therefore, this interaction has wide applications, including the study of

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genetic diversity and finding the genetic footprint, as well as in the field of breeding and improvement of animals and plants. This technology was used to facilitate the results of crossbreeding and hybridization when trying to devise new varieties, as well as used in the field of diagnosing various diseases such as genetic and epidemiological diseases (Hassan, 2011).

Single Nucleotide Polymorphism (SNP):

They are indicators that are no less important than the previous genetic indicators and are defined as indicators of the third generation (Wang et al., 1998). Thus, it leads to an allelic difference. The difference in the sequence of the nitrogenous bases has been known since 1977 when the base tracking technology (DNA sequencing) was implemented, but the possibility of determining the genetic structure based on the polymorphism of the single nucleotide SNP for a large number of samples was not available until the application of the genetic chip technology (Gene chip) was implemented at the end of the year 1990. The importance of SNP emerged because it is appropriate to show the phenotypic diversity that cannot be reached by other types of indicators, the SNP of one genetic locus results in four alleles due to the presence of four nitrogenous bases, but in reality only two alleles are produced, so it is considered bi-allelic and is according to the co-dominance (Lui and Cordes, 2004). Genetic variation occurs at the DNA level as a result of a difference in one of the nitrogenous bases of the gene (Promoter, Intron, Exon) or between genes (intergenic regions) and this difference results from substitution, deletion, or addition (Supek et al., 2014 and Nadeem, 2018) and can only be detected by the availability of specialized primers in a specific region of a specific DNA gene called a single nucleotide polymorphism (SNP) (Van Goor et al., 2015). The nature of the genetic mutation is what determines the nature of the single nucleotide polymorphism (SNP) and it is in two forms, the first of which is mutations that replace a nitrogenous base with another of the same type, purine with purine such as (adenine in place of guanine) or primidine with pyrimidine such as (thymine in place of cytosine) called in this case Transition. As for the second type, a nitrogenous base is replaced by another type of purine with pyrimidine, such as (adenine in place of thymine or guanine in place of cytosine and this mutation is called Transversion (Wang et al., 1998 and Lorenc et al., 2012). SNPs are classified within the genetic regions that encode mRNA into two types, the first type is a synonymous mutation that occurs as a result of changing a single base pair without resulting any change in the translated protein resulting from gene expression or may have little effect on gene expression, while the second type is known as a non-synonymous mutation that leads to change in amino acid, and this is more important than the first type due to its potential effect on gene expression and the formation of a protein different from the usual protein, and this in turn will affect the external appearance of the organism, so, both synonymous and non-synonymous SNPs are important as a genetic indicators in the genetic mapping study (Emara and Kim, 2003). These indicators become dominant form of molecular indicators used in genetic analysis, as they are considered genetic indicators that describe genetic changes as a link between phenotype and genotype, as it was noted that there is one SNP for every 1000 to 2000 bases within the DNA and in some regions it is estimated to be one SNP per 300 bases (Dawson, 1999).

Restriction Fragment Length Polymorphism (RFLP):

It is one of the techniques used to detect genetic markers and is symbolized by the symbol RFLP. It is a technique that depends on the different lengths of the DNA resulting from the use of specific restriction enzyme. This technique can be used in mapping the genotype and DNA (Beattie, 1994). Fulton (2014) indicated that the RFLP technology is one of the most important techniques that developed DNA maps resulting from genetic variation, which was identified by using restriction cutting enzymes, and then it was commonly used by commercial companies to detect genetic variations and, genetic fixation and gene determination. Genetic variation occurs at the DNA level as a result of the difference in one of the nitrogenous bases of the gene (Promoter, Intron, Exon) or between genes (regions between genes) and this difference is caused by replacement, deletion or addition (Nadeem, 2018) and cannot be detected only by the availability of specific primers to a specific region of a particular DNA gene and the use of RFLP technology is one of the techniques used to detect those differences in the DNA strand by restriction enzymes which results in different lengths of the genetic region under study resulting from a genetic mutation in one of the allele locus responsible for the trait studied (Supek et al., 2014). The RFLPs

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technique was used to develop the genetic map and to determine the genetic locations of the quantitative traits QTL in agricultural animals (Beattie, 1994), but the high cost and time required to implement this technique limited its widespread use in the poultry industry. However, the genetic information that was disclosed by using this technique, commercial breeding companies were interested in starting direct examination to detect genetic variations at the DNA level within breeding flocks, this led to the establishment of the global chicken breeding company Hy-Line International, which was established through molecular genetics techniques using RFLP technology. Soller and Beckmann, (1985) showed, the use of RFLP technology is a great benefit in breeding programs, especially when applied to economic traits in poultry.

Conclusion:

The search and investigation of the genotypes possessed by birds characterized by high productive performance is important in sustaining selection by searching for marker assisted selection (MAS), especially single nucleotide polymorphism (SNP), which is related to productive traits (Teneva, 2009) Therefore, the use of these techniques is considered a more efficient way to study productive traits in chickens and has become increasingly important in poultry breeding and selection programs.

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