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Section I: Agricultural and Animal Biotechnology

Mini Review

Genómica y producción animal

Genomics and animal production

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Abstract

Developing countries have the challenge of achieving food security in a world context that is affected by climate change and global population growth. Molecular Genetics and genomics are proposed as technologies that will help to achieve sustainable food security. Technologies that have been developed in the last decade such as the development of genetic markers, genetic maps, genomic selection, next-generation sequencing, and DNA editing systems are discussed. Examples of some discoveries and achievements are provided.

Resumen

Los países en vías de desarrollo tienen el reto de alcanzar seguridad alimentaria en un contexto mundial afectado por el cambio climático y crecimiento poblacional global. La genética molecular y la genómica son propuestas como tecnologías que ayudarán a alzanzar una seguridad alimentaria sostenible. Tecnologías que han sido desarrolladas en la última década como el desarrollo de marcadores moleculares, mapeo genético, selección genómica, secuenciamiento de próxima generación y sistemas de edición de ADN son discutidos. Se proveen ejemplos de algunos descubrimientos y logros.

Palabras clave:

Marcadores genéticos; mapeo genético; selección genómica; NGS; sistemas de edición de ADN.

Keywords:

Genetic markers; genetic maps; genomic selection; NGS; DNA editing systems.

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Introduction

For developing countries, the challenge of achieving food security is probably much more complex and discouraging than it was in the last century. We now have a world of approximately 7 billion inhabitants in which it is estimated that 1 billion are undernourished and a similar number are overnourished. This lack of food for some and excess for others creates serious human health problems that dominate the agriculture agenda and world health constituting one of the greatest global challenges. This situation is aggravated by the fact that world population in 2050 is estimated to reach 9 billion inhabitants and, according to estimates of the United Nations, there would be a need for 35% more food, 40% more water and 50% more energy to sustain this population. These increases will have to be achieved in a short period with almost the same arable area that now exists in a world that faces climatic changes affecting the availability of water for agriculture and human consumption among other challenges. Clearly the future demands better coordination and collaboration among nations, better distribution of food resources within and between nations, and sustainable intensification of agricultural production at a global level. We believe that the advances achieved over the last 10 years with molecular genetics and genomics can be used to develop more productive, and efficient livestock production based on genetic improvement, the use of reproductive technologies and DNA editing. The purpose of this manuscript is to provide information on some of these advances and suggest their use to develop sustainable livestock farming.

Genetic markers

One of the first goals of production animal genomics was the development of genetic maps based on molecular markers. The most used markers have been microsatellites (MS) and single nucleotide polymorphisms (SNP). The former are unique sequences of DNA flanking a fragment containing tandemly arranged di, tri, tetranucleotide repeats or combinations of them. The variation in the number of repetitions creates fragments of different sizes and hence alleles. The unique sequences flanking the MS are used to design primers that allow amplifying the MS by the polymerase chain reaction (PCR). Amplified fragments are visualized by DNA electrophoresis. An MS can have from two to more than 15 informative alleles. Informative alleles are those that have at least 0.05 frequency in a population.

SNPs instead are substitutions of one nucleotide by another at a specific position within a fragment of DNA in which some individuals of the population have one nucleotide and other individuals have a different nucleotide at the same position. Although SNPs can have a maximum of four alleles, mostly only two are observed in any specific population. A marker with only two alleles is a marker that has very low resolution. However, due to the abundance of these markers, groupings of markers along a stretch of DNA can be made, thus establishing haplotypes (a specific set of markers consecutively ordered in a fragment of DNA) that are used to do studies of their association with quantitative trait loci. The method of detection of the SNPs has been automated in such a way that one can make a simultaneous analysis of thousands of these markers in a single reaction for each animal. In this manner, one can analyze SNP genotypes for every animal, in a sample, covering more than 95% of their genome.

Genetic maps

Molecular genetic markers are generated using different strategies. Microsatellites are isolated from libraries of short DNA fragments using probes constructed with tandem repeats of 2, 3 or 4 nucleotides followed by sequencing of the detected fragments to identify unique site sequences flanking the MS to design PCR primers. SNPs are identified by sequencing short fragments of DNA and comparing sequences of similar DNA fragments among animals of the same species. However, the localization of markers along the length of DNA in a genome cannot be known unless genetic marker maps are built. The technology to develop these maps is based on the analysis of linkage between genes and/or genetic markers that was described by Sturtevant (1913) when he built the first chromosomal map at the beginning of last century. Two genes are linked (close) in the same DNA strand when the recombination frequency between them is low. At a lower distance between two genes or markers, the lower the likelihood of recombination or chiasma bridges that can be generated between them during the meiotic cell division. To develop these maps researchers had to generate appropriate crosses between animals that resulted in the highest proportion of informative meiosis. Pseudo-genetic analysis systems like Radiation Hybrids were also used to facilitate the sequential ordering of the several thousands of molecular markers that exist for each species. We currently have genetic maps of SNP markers for ruminants (bovine, buffalo, sheep, and goat) non-ruminant (rabbit, pig, horse), poultry (chicken, turkey, duck) among other farm animal species. Some maps are more informative than others are because they contain more markers with average distances between them ranging from 7,500 pairs of bases (pb) to 100,000 pb. In one way or another, these maps have facilitated the ordering of DNA fragments (physical maps) obtained from genomic DNA libraries facilitating in this way the ordering of DNA sequences obtained from the DNA sequencing projects of farm animals. For more information on the sequenced species, see the pages of the National Center for Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov/).

Molecular markers are used for evolutionary analysis, gene flow, genetic variability, population genetic structure analysis, detection of DNA-specific sequences, genetic improvement by assisted selection, controlled mating system, identification of individuals and paternity analysis among other uses.

SNP "Chips"

Depending on the number of SNPs available for each species, SNPs that were distanced between them by 3,500 bp (cattle) to 54.000 bp (chicken, sheep, pork)

among them were chosen to generate SNP chips. Synthesized DNA fragment sequences containing each of these SNPs are generated, spotted and fixed on a solid support as microscopic points. Each of these microscopic dots contains a specific sequence of an SNP that can be detected by hybridization to DNA fragment sequences of the individual animal being genotyped (Affymetrix) or by hybridization and extension of the SNP sequence based on a DNA guide of the Animal being genotyped (Illumina). The number of SNPs on the chip can be designed at will depending on the availability of SNPs and the degree of resolution desired. The greater the number of SNP, the lower the distance between them on a genome and the greater the resolution and precision of identification of genes or segment of DNA associated with phenotypic characteristics. Most research on phenotypic associations with markers is made with SNP chips containing between 50,000 and 60,000 SNPs.

Our research group is focusing in finding a large number of SNP in alpacas to build a beadchip for this specie. Three strategies have been carried out, first using an HD bovine beadchip (Mamani et al. 2017; More et al. 2019), second using comparative genome analysis among genes related to fiber growth of sheep, goat and alpacas (Fernandez et al. 2019), and third using sequences of DNA reduced libraries from 150 alpacas and 6 sequenced genomes at 30X depth (Unpublished data). In addition, some genes and SNPs have been localized, by fluorescent in situ hybridization (FISH) on alpaca chromosomes (Mendoza et al. 2019). Since the size of the alpaca genome is about 2.1 x 10^9 bp, we seek to select about 50,000 SNPs, each one separated by 40,000 bp, and covering about 90% of its genome.

Quantitative Trait loci (QTL) mapping

QTL is understood as a specific chromosomal region that has been identified, by statistical methods, to be associated with a quantitative phenotype. The region itself could contain one or more genes responsible for the genetic control and segregation of the phenotype. Fundamentally, the principle on which the identification of a QTL is based is to detect the linkage imbalance, disequilibrium, that exists between a gene or genes that control a quantitative variable and a marker or molecular markers in the genome. The availability of SNP chips and databases with quantitative information of phenotypes has allowed identifying QTLs for most of the phenotypic traits under genetic improvement in some species. In the "AnimalQTLdb" (Http://www.animalgenome.org/cgi-bin/QTLdb/index) (Zhi-Liang et al. 2013) database one can find more detail about specific QTL for farm animal species. It is sufficient here to highlight that 11,543 QTLs representing 481 production traits have been identified in bovine; 4,337 QTLs representing 305 traits in chickens; 11,610 QTLs representing 649 traits in pigs; 789 QTLs representing 217 traits in sheep; 345 QTLs represented 9 traits in equine and 127 QTLs representing 14 traits in trout, have been identified. This information is being used to estimate the genetic value of an animal based on the association of markers with productive traits (Meuwissen, et al. 2001).

Genomic selection in dairy cattle

The approximately 38,000 informative SNPs that are associated with traits of dairy production are distributed throughout the genome of each animal and theoretically, the space between each of them is approximately 80,000 nucleotides. This means that the SNP markers are consecutive and are relatively close to each other. The parents transfer this ordering to their descendants. The descendant, due to the recombination of maternal and paternal DNA occurring in its reproductive cells, transfers to the new descendant generation (or grandchildren of the evaluated parents) fragments of the grandfather's DNA mixed with fragments of the grandmother's DNA. The genetic information that is sought in the genomic evaluation of each animal is the identification of the fragments of DNA that have been inherited and that are positively or negatively associated to the traits of production to be improved. This is accomplished by analyzing the SNPs that each animal inherits. Thus we obtain a map of SNP markers for each animal or the genotype of each animal and then it can be determined which DNA fragments the animal has inherited from its ancestors which in turn have been evaluated by progeny tests or by genomic evaluation. Based on studies of the association of markers and productive traits made in a reference population, specific values are allocated to each inherited fragment and the genomic PTA (GPTA) is obtained for each trait under evaluation. I think it appropriate to remember here that the consecutive ordering of SNP markers found in a fragment of DNA is known by the name of "Haplotype". Therefore each haplotype is given a value that represents its contribution (positive or negative) to the productive trait to which it is associated. The reliability of this estimate has been assessed and has been determined to be superior to the reliability of the PTA. Van Raden et al. (2009) concluded that the average reliability obtained for 28 production traits assessed in the U.S. Holstein breed was 23% higher than the average reliability based on the average parent PTA. This advantage is equivalent to evaluating the production of eleven daughters. Reliability increases more by doubling the number of bulls evaluated than by increasing the number of SNP markers. These conclusions were obtained after using the evaluations obtained in August of 2003 from 3,576 Bulls born before 1999, which served to predict the deviations of production of the daughters of 1,759 bulls born between 1999 and 2002. For example, the reliability for milk production based on the average of the parents was 28% and reliability based on non-linear genomic prediction was 49%, indicating a 21% advantage. The trait that presented the greatest advantage was the percent of fat in which the average of the parents PTA reached 25% and the genomic prediction was 63%. The main reason for this result is the existence of a gene with a major effect. Similar evaluations and results have been obtained in Australia, France, and New Zealand which encourages the use of GPTA in bulls 2 years old or less which significantly reduces the generational interval and allows doubling annual genetic gain (Hayes et al. 2009). In the same way in other studies, VanRaden et al. (2011) identified

five haplotypes that are not in homozygote state in the population of genotyped animals of the Holstein breed and have a negative effect on the percentage of conceptions when haplotypes are segregated by the father and maternal grandfather. The percentages of animals carrying these haplotypes vary between 2.7 to 6.4%. The genes that produce this negative effect are not yet known, and one can only infer that in the DNA segments identified by these negative haplotypes there are some genes that when in homozygous condition cause losses by low fertility or early embryonic death. The existence of these negative haplotypes does not mean that the carrier animals should be eliminated as future progenitors. Rather, the available information should be used to program controlled crosses that do not allow the possibility of generating homozygous individuals. Other examples of this nature exist in the scientific literature and the conglomerate of industry-related institutions and companies are supplying the information to producers as soon as they identify detrimental or negative haplotypes.

Genomic selection in other species

Van Eenennaam et al. (2014) has published a review on the pragmatic use of genomic selection in cattle (dairy and beef), pigs and chickens. These authors conclude that the genomic selection in dairy cattle is successful due to the existence of phenotypic data banks accumulated through several decades of progeny testing that allow achieving significant levels of accuracy and reliability. In sum, the reference populations already exist in the databases. However, it is assumed that the degree of adoption and use of genomic selection by other livestock and poultry industries will be influenced by:

1. Biological limitations of the species,
2. The organizational structure of the industry,
3. Determination of the ideal size of the reference populations that represents a significant investment,
4. The development of economic and efficient genotyping strategies,
5. The practicality of its implementation in the field,
6. The cost of genomic selection compared to the benefits obtained by the actual annual genetic gain.

For example, the genotyping of SNPs with high-density SNP chips in selected males and low density in females is being used successfully to impute genotypes in the descendants making genomic selection economically more efficient for the pig and poultry industry

New generation sequencing technologies

In the last 8 years, the development of technologies of new generation sequencing (NGS), also called parallel massive sequencing allows to obtain between 1 million and 43 million reads (each reading is a small fragment of ~ 50 bp to 400 bp) per sequencer instrument in one run depending on the sequencing platform used. The sequencing cost has also dropped significantly from \$9.00 per mega-base in the year 2001 to approximately \$0.08 per mega-base in the year 2014 (National Human Genome Research Institute http://www.genome.gov/sequencingcosts/). This makes it possible, at a reasonable cost, to carry on studies comparing genomes between animals within a breed, between breeds and among species to detect genetic variation at the gene level and in gene-regulating sequences. These technologies are applied in the de novo sequencing of genomes, re-sequencing of specific areas of the genome, the discovery of genetic markers, discovery of DNA structural variation, sequencing transcriptomes, sequencing of methylomes, the discovery of RNA variants, identification of non-coding RNAs (ncRNAs), etc.

These technologies are revolutionizing our understanding of what is genomic variation and allow us to discover the genetic mechanisms that govern the expression of genes and their phenotypes that previously we could only measure quantitatively or qualitatively. For example, recently Pausch et al. (2014), based on genomic research and NGS deep sequencing, found in the Fleckvieh breed a meaningless mutation in exon 6 of the gene that encodes the transmembrane protein 95 located in a normal spermatozoid membrane. The mutation of a single nucleotide change (SNP) creates a premature termination codon with which the protein is truncated and non-functional. This protein is not present in spermatozoa of recessive homozygous animals resulting in a significant reduction in fertility. In the same way in cattle of the Piedmonts breed, the mutation of a nucleotide (SNP) in the myostatin gene causes muscular hypertrophy that is known as the "double musculature" phenotype (McPherron & Lee, 1997). The animals that present this phenotype have, on average, 20% more muscle mass. In the first example, the gene of the transmembrane protein 95 would be an ideal candidate to be corrected in elite animals of the breed that present advantages in other traits. In the second example, the mutated gene of myostatin could be a candidate gene for introgression to other breeds.

DNA editing technologies

One of the ways to assess the function of a gene is by altering the gene sequence or by silencing the gene activity in some way and observing changes in the phenotype. For this purpose, systems have been developed that use enzyme nucleases to make changes in the DNA sequence that allow to either repair or mutate a gene or its regulatory sequences. These nucleases are enzymes that are known as "molecular scissors" because they cut the double DNA chain at precise places. The precision of the recognition of a specific sequence of DNA by the enzyme is achieved by using a guide and then use the natural endogenous homologous recombination mechanisms of the cell to repair the DNA and copy the sequence stipulated in the guide as part of the repaired fragment. These technologies are considered the most important in recent years because they will be able to do what is now known as "genetic surgery" that consists of the directed editing of DNA sequences in living cells. Currently, three nucleases are most often used that are designed to recognize specific DNA sequences for editing.

The first nucleases ever used to edit DNA are the "zinc finger nucleases" (ZFN). These nucleases consist of a chain of approximately 30 amino acids that recognize their nucleotide triplets. Currently, ZFNs have been designed to recognize the 64 combinations of triplets that can be found in DNA. With this researchers can build dimers that recognize 6 bases, or two triplets, and in this way, they can prepare dimers that recognize a sequence at the 5 'end of the sequence to be edited and another at the 3 ' end (Carroll 2011).

The identification of nucleases known as TALENS (Transcription Activator-like Effector Nucleases) that are similar to transcription-activating enzymes act in a similar way to ZNFs. These use amino acid blocks to recognize specific nucleotides so that assembling several blocks in a specific order can recognize a specific sequence in the DNA. It is then necessary to design a TALEN to recognize a specific sequence at the 5 ' end and another TALEN to recognize a sequence at the 3 ' end of the sequence that one wants to modify (Joung & Sander 2013).

The most recent nuclease used to edit DNA is the one that recognizes clustered regularly interspaced short palindromic repeat sequences (CRISPR) in the DNA known as Cas to generate the CRISPR/Cas system. This system is based on the use of a small molecule of RNA that serves as a guide to recognize the specific sequence of DNA to be edited. The RNA guide molecule (gRNA) consists of a part that recognizes the enzyme and another of approximately 20 nucleotides long that recognizes the DNA sequence to be edited. The latter forms an enzyme/RNA complex that localizes the DNA sequence to be edited and binds to it (Sanger & Joung 2014).

Of the three systems described the one that is most used is the CRISPR/Cas system with which it has been possible to modify genes in living cells and to produce animals and plants carrying the gene modifications. Genetic errors have also been corrected by direct infusion of CRISPR/Cas-RNA guide complexes in adult animal organs. For example, Yin et al. (2014), through hydrodynamic injection of the components of the system CRISPR/Cas, were able to correct the expression of the gene Fah in ~ 1/250 cells of the liver of adult mice. The expansion of Fah positive hepatocytes rescued animals from the weight loss observed when the Fah gene is not functional. This system also allows editing several loci at a time in a single embryo. Simplicity, high efficiency and its breadth of applicability will allow designing experiments that are more complex in order to elucidate interactions between genes, something quite difficult to do so far. Of course, these systems are under assessment and improvements to increase their accuracy and determine their effect on the genome. In the future, cloning techniques (Wilmut et al. 1997, Cibelli et al. 1998), precise DNA genomic sequencing information and editing of DNA will be synergistically used for genetic improvement by introducing beneficial alleles of a breed into the genomes of elite animals of other breeds. The latter will avoid the introduction of unwanted genetic material that accompanies the introgression of alleles by traditional crossbreeding (Tan et al. 2012).

Conclusions

Progress in genomics and molecular genetics has proved useful in the development of genomic selection. This has been possible due to the development of molecular markers that have allowed identifying segments of the genome that contain genes controlling quantitative traits. The application of genomic selection in dairy cattle is, for the moment, the most successful. The use of genomic selection in other species is evolving and will depend on the breeding structure and genetic improvement being applied to those species. Its use will also depend on the costs of the technology. Most farm animal species already have sequenced genomes at different levels of depth, but in general, the level of sequencing achieved is already informative and due to the constant lowering of the costs of sequencing technology it is envisioned that increases in sequencing resolution will continue to improve. The new technologies of cloning and DNA editing, if they have social acceptance, coupled with the knowledge of gene sequences and their allelic variants will allow the "molecular introgression" of beneficial alleles between breeds and the correction of detrimental mutant alleles. These techniques could also help reduce the environmental footprint of the livestock industry. The reduction of the generation of methane, one of the gases that contribute to the greenhouse effect, in cattle is obtained by producing more product (milk or meat) per animal or it is obtained by modifying the flora of the rumen. Genomic selection could contribute very quickly to the increase in milk production per animal as well as help to understand the composition of the rumen flora. On the other hand, the modification of the myostatin gene in meat producing breeds through genetic editing systems could increase meat production by 20% per animal. In both cases, the volume of methane produced per unit of product (liters of milk or kilos of meat) would be lower than the current one.

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