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Review

The role of molecular genetics in livestock production

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Abstract. Genetic variations that lead to easy-to-identify phenotypic changes have always been of interest to livestock breeders since domestication. Molecular genetics has opened up possibilities for identifying these variations and understanding their biological and population effects. Moreover, molecular genetics is part of the most diverse approaches and applications in animal production nowadays, including paternity testing, selection based on genetic variants, diagnostic of genetic diseases, reproductive biotechniques, fraud identification, differentiation of hybrids, parasite identification, genetic evaluation, diversity studies, and genome editing, among others. Therefore, the objective of this review was to describe the different applications of molecular genetics in livestock production, contextualising them with examples and highlighting the importance of the study of these topics and their applications.

Additional keywords: animal husbandry, genetic variation, molecular markers.

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Introduction

In domestic animals, the first evidence of mutations was obtained by phenotypic observation, i.e. the direct contact led to the observation of the occurrence of animals with a different phenotype that could be transmitted to their offspring. Some of the earliest examples are five-toed Houdan birds, polled Hereford cattle, and short-legged Ancon sheep (Domingues 1957). From the first discoveries of genetic mechanisms, researchers tried to infer about the inheritance of these traits in domestic animals, often based on other living beings.

The action of man through domestication and phenotypic selection has altered the genetic constitution of livestock populations. An example is the allele fixation of genes related to the behaviour of rabbits (Carneiro *et al*. 2014), which were selected for docility during domestication. Nowadays, with the development of molecular genetic techniques, it is possible to 'visualise' alterations in hereditary molecules and to understand spontaneous variations, their phenotypic effects and their transmission to descendants. Moreover, molecular genetics plays an important role in animal production, permitting paternity testing and the identification of frauds and genetic diseases, among other utilities that will be addressed in this review.

Therefore, the objective of the present article was to report the different perspectives of the application of molecular genetics not only to animal breeding and genetics, but also to the livestock production, providing examples of successful and recurrent use, in addition to better contextualisation of this information by demonstrating how to use it.

Selection based on genetic variants

When we talk about molecular genetics applied to animal production, we immediately think of the identification of genes, their polymorphisms, their phenotypic effects and consequently their possible use in selection. There are two main applications of genetic variants: selection based on a functional mutation and the inclusion of this genetic variant in the model used for genetic evaluation.

Causal mutations are polymorphisms responsible for trait variation (Hayes *et al*. 2014). Selection based on a causal mutation is used in the case of a qualitative trait or when the mutation explains a large portion of the additive genetic variance in a trait (major gene). There are some examples in livestock: (1) selection for polledness in cattle, which apparently has dominant Mendelian genetics. Although the causal mutation has not yet been discovered, indirect molecular markers can be used to select polled animals. Homozygous polled animals are distinguished from heterozygous animals and selection is done more efficiently (Mariasegaram *et al*. 2012; Wiedemar *et al*. 2014). The objective of selecting for polled animals is the easier management of these animals, which do not require dehorning to prevent them from getting hurt (Schafberg and Swalve 2015; Mendonça *et al*. 2016). (2) Coat colour of horses: this species is characterised by wide variation in coat colour. The effects of the genes involved are phenotypically observable and it is therefore interesting to identify the genes that act on this trait and to develop molecular markers. Producers have preferences for certain coats and restrictions even exist for some breeds. Many matings are performed to obtain certain coats

(Sponenberg and Bellone 2017). (3) Production of A2 milk: one of the proteins in cow's milk is β-casein. The β-casein gene has two alleles, A1 and A2, among others. The A1 allele produces the β-casein A1 in milk, which has been shown to be the cause of milk allergy seen in some people (Jianqin *et al*. 2016). The milk of animals whose genotype is A2A2 of the β-casein gene, is called A2 milk, contains only β-casein A2 and can be consumed by these allergic individuals. The difference between alleles is due to an A/C SNP in exon 7, which causes a change in the encoded amino acid from histidine to proline. Cows with the A2A2 genotype can be identified by using molecular markers (Olenski *et al*. 2010).

There are other polymorphisms that explain a large portion of the additive genetic variance in traits and that can be used in marker-assisted selection. The *RYR1* gene (halothane gene) codes a calcium-release channel protein. A mutation in the gene of this receptor causes malignant hyperthermia syndrome, also known as porcine stress syndrome, which leads to pale, soft and exudative meat in pigs. In this species, a C/T SNP exists at position 1843 of the gene that changes the amino acid at position 615 from arginine to cysteine. Individuals with TT genotype (recessive homozygote) have pale, soft and exudative meat that cannot be commercialised. Thus, marker-assisted selection is performed to avoid animals that carry the allele T (Rojas *et al*. 2008). Another example is the *BMPR-IB* gene, which is related to prolificacy in sheep. An A/G SNP at position 476 of the gene causes an amino acid substitution at position 249 from glutamine (allele A) to arginine (allele G) (Mulsant *et al*. 2001). Selection for allele G is performed to increase the prolificacy of sheep herds.

It must be remembered that in the above cases the culling or non-use of animals with unfavourable genotypes/alleles for breeding should not be radical. Animals can have unfavourable genotypes for a certain trait, but may have favourable genotypes for another trait. Selection based on the information of one locus or few loci may result in considerable loss of genetic variability.

Another possibility of working with genes of known effect is the inclusion of the effect of the genotype in the genetic evaluation model as a fixed effect (Kennedy *et al*. 1992). The inclusion of the causal mutation is beneficial for genetic evaluation as it adds important information to explain variation.

Paternity tests

Paternity tests in domestic animals have two main applications: registering animals in breed associations and providing accurate pedigree information for genetic evaluations. Some breed associations require paternity test for registration, whereas others randomly select animals to be submitted to paternity testing for registration.

In genetic evaluations, the pedigree among the animals evaluated is of fundamental importance for the prediction of breeding values. Incorrect relationships can lead to prediction errors. Nowadays, with the advent of genomic selection, paternity errors are easily identifiable, as the SNP array genotyping used for selection may be also used for parentage testing.

For domestic animals, the molecular genetic markers used in relationship tests are evaluated and accredited by the International Society for Animal Genetics (ISAG). The mechanism of validation of paternity tests developed by researchers is described in detail at the website of the society (http://www.isag.us/comptest.asp, accessed 3 June 2018). In summary, the results found by researchers are submitted to ISAG, a committee evaluates the results, and other accredited laboratories repeat the tests for their validation. Quality standards are applied to assess and confirm the results and the rules are determined according to the type of marker used (microsatellite, SNP, other). Several examples of validated paternity tests for different livestock species can be found at the ISAG website and are always presented at the meeting of the society (http://www.isag.us/committees.asp, accessed 3 June 2018).

Genetic diseases

There are series of genetic diseases that affect domestic animals and therefore influence animal production. The identification of genetic variants of these diseases permits to control and guide matings so that the damage caused by the disease is not propagated.

An example of a disease in livestock with direct implications for animal production is hyperkalemic periodic paralysis in horses. This is a dominant autosomal genetic disorder that is important for Quarter Horses and related/derived breeds. Symptoms of the disease include episodes of weakness, tremors and intermittent paralysis (Rudolph *et al*. 1992). The animals also exhibit a phenotype of hypertrophied muscles. This phenotype was highly desired by breeders of the conformation line of Quarter Horses, which led to the spread of the disease together with its detrimental effects.

Rudolph *et al*. (1992) identified an SNP in the *SCN4A* gene as the genetic cause of the disease. The gene regulates the transport of sodium in muscles. The SNP consists of a nucleotide substitution of cytosine (C) to guanine (G). The fact that the SNP is located in a codifying region results in an amino acid change from leucine to phenylalanine. The altered protein prolongs the period of sodium channel opening and results in the phenotype.

Today, many Quarter Horse breeder associations request a genetic test for the disease and do not accept the registry of animals homozygous for the mutated allele or heterozygotes. It is therefore a practical example of a molecular marker for diseases that is applied in animal production.

In cattle, BLAD (bovine leucocyte adhesion deficiency) disease is an autosomal recessive lethal disease. The affected calf suffers from different infections and dye. The causing genetic variant is a SNP A/G located in *CD18* gene that modifies aspartic acid to a glycine at amino acid position 128 (Shuster *et al*. 1992). Although the mutation was discovered some time ago, new genotyping techniques have been developed for the SNP (Alyethodi *et al*. 2018). There is a necessity to report sires genotype in order to identify potential carries for bull catalogues.

Reproductive biotechniques

Reproduction is fundamental for the viability of production systems. Moreover, reproductive biotechniques enabled the development of other areas of animal production such as

animal breeding and genetics. The advent of artificial insemination permitted the accurate genetic evaluations of males and the dissemination of superior genetic material. Multiple ovulation, *in vitro* fertilisation and embryo transfer have improved the dissemination of genetic material of females.

Molecular genetic techniques potentiate the effect of reproductive biotechniques and consequently the profitability of the intensive livestock sector. Successful examples are the use of sexed semen and embryo sexing to increase the production of males or females as desired. Flow cytometry separates spermatozoa bearing the X or Y chromosome based on the amount of fluorescence emitted by stained DNA exposed to laser irradiation. Spermatozoa carrying the X chromosome have more DNA and therefore emit more fluorescence (Espinosa-Cervantes and Córdova-Izquierdo 2012). After sexing of gametes, genetic tests are performed to validate the efficiency of the technique and to ensure the use of sexed semen. Parati *et al*. (2006) developed a quantitative polymerase chain reaction (PCR) technique for sexing spermatozoa in cattle. Sex chromosome-specific primers and probes were synthesised. The determination of threshold cycle values for X and Y chromosomes probes indicated efficiency of sperm sexing. Many other techniques have been developed for the same purpose.

The same approach of sexing can be applied to embryos produced *in vitro*, in which only embryos of a given sex are implanted because production has a greater interest in that sex. Khamlor *et al*. (2015) developed a marker based on loop-mediated isothermal amplification (LAMP) technique where fluorescence-labelled specific primer pairs are used for sexing bovine embryo and Tavares *et al*. (2015) developed a multiplex PCR (specific primer pairs for sex chromosomes) for bovine and sheep embryo sexing. The main obstacle of embryo sexing is the amount of DNA that needs to be extracted without damaging the embryo. As the quantity of DNA extracted is very small, these techniques are preceded by a nested PCR, i.e. before sexing itself, a larger DNA region that comprises the region to be used for sexing is amplified employing outside primers. Thus, a sufficient amount of DNA to ensure execution of the subsequent technique is obtained.

Fraud identification

Molecular genetic markers are very useful to identify possible frauds in animal products such as meat, milk and their derivatives. This type of fraud is common to reduce production costs; for example, cow's milk is mixed with the milk of other species (goat, buffalo or sheep) and meat and meat products of domestic mammals (cattle, sheep, goat, pig and horse) and domestic birds (chicken, turkey). Frauds can be identified by protein analysis, but DNA tests have been shown to be cheaper and equally or even more effective (Di Domenico *et al*. 2017).

Tests using genetic markers can provide purity assurance to the consumer to avoid deception. Di Domenico *et al*. (2017), using species-specific probes and real-time PCR, amplified partial regions of mitochondrial DNA and rRNA genes that permitted to rapidly and efficiently differentiate mixtures of cattle, buffalo, sheep and goat milk. Ghovvati *et al*. (2009) and Kesmen *et al*. (2009), employing multiplex PCR and real-time PCR probes, developed tests that were able to identify possible frauds that mixed ruminant, poultry and pork meat and horse, donkey, cattle, pork, sheep, chicken and turkey meat, respectively. The tests were based on primers targeting specific regions of the rRNA genes of the species. Many of these fraud tests are carried out using these gene regions because of the large amount of mitochondrial DNA/rRNA in cells and the great interspecific variability.

Genetic markers can also be used for traceability. Arana *et al*. (2002) developed a set of microsatellite markers to certify the origin of beef cuts. It is a simple test that can be used to increase consumer confidence and to add value to the product. Arana *et al*. (2002) established that a minimum of eight markers with a high degree of heterozygity is necessary for valid identification as well as knowledge of the population structure. The main limitation would be the implementation cost.

Identification of hybrids

Genetic contamination (fish)

A very recurrent practice in fish farming is the use of hybrids (crossbreeds of different species) for production. These animals are characterised by rapid growth and finishing, which is mainly due to the extreme heterosis of hybrids resulting from the gene combination of different species.

One example is the hybridisation that exists between three species of the family Serrasalminae (*Colossoma macropomum*, *Piaractus mesopotamicus* and *Piaractus brachypomus*). Some hybrids can be fertile and backcrossing of hybrid individuals with the parental generation can lead to genetic contamination of pure populations. This results in serious production problems that range from the loss of heterosis to low incubation rates and high mortality rates, hampering reproductive performance, with negative consequences for the productivity and profitability of rural producers (Hashimoto *et al*. 2012).

To partially control this problem, Hashimoto *et al*. (2014) developed molecular markers based on multiplex PCR and PCR-RFLP for the identification of pure and hybrid animals. The techniques are based on the amplification of fragments that differ in size between species through the combined use of various primer pairs and their annealing or not (multiplex PCR) or due to the formation of species-specific fragments of different sizes by restriction enzymes (PCR-RFLP). This is a well-defined application of molecular genetics to animal production, which permits through genetic analysis to select pure animals for reproduction in order to avoid the risks resulting from genetic contamination.

The legislation of many countries prohibits the use of hybrid fish because of the risks involved. However, some researchers have tried to overcome this problem because of the advantage of heterosis, which is interesting for production. This solution is also based on genetic concepts: the production of triploid individuals (3n), i.e. animals with three haploid sets of the genome, which are viable in the fish class. Triploid fish would be infertile, thus permitting the production of triploid hybrids that do not reproduce or cause genetic contamination. The production of 3n zygotes is achieved by inhibiting expulsion of the polar body of the second oocyte by thermal shock after external

fertilisation. Thus, the egg is 2n and fecundation with spermatozoa n gives origin to a 3n individual. Nascimento *et al*. (2017) induced triploidy in lambari (*Astyanax altiparanae*). Triploid organisms show better performance than diploid individuals. Triploid females are sterile, but triploid males continue to be fertile. Further studies are needed to increase our understanding of the technique and its application to commercial species.

Equids

Equids include two domestic species: horses (*Equus caballus*) and asses (*Equus asinus*). The hybrids among equines are very easily confused. Two possibilities of hybrids between these species exist: the cross of a mare and a male donkey results in a mule, whereas the cross of a stallion and a female donkey results in a hinny. The hybrids of the two crosses are easily confused because of their close phenotypic similarity. Moustafa *et al*. (2017) and Franco *et al*. (2016) developed mitochondrial DNA markers that can distinguish these hybrids. Using PCR-RFLP, Moustafa *et al*. (2017) amplified the same region of Cytochrome *b* in both species (horses and asses). Restriction enzyme cleavage produced distinct migration patterns, with hinnies exhibiting the same pattern as asses and mules the same pattern as horses as they share the same mitochondrial DNA. Franco *et al*. (2016) used multiplex PCR, i.e. a specific primer pair for each species. Both primers amplified the D-loop of mitochondrial DNA but the size of the amplified fragment varied according to species and the hybrids could be identified by observing the size of the DNA band on a gel.

Parasite identification

Parasitology is an important area in animal production. There are high costs related to preventive practices. Moreover, inefficient sanitary management can lead to a decline in productive performance as well as morbidity and death of the animals. High rates of infestation require the application of specific anti-parasitic agents. The intensity of application and drug of choice should be appropriate in order to avoid the selection of resistant parasite populations. The available techniques such as faecal egg count, larval culture or microscopic examination are poorly sensitive and are time consuming. Thus, the efficient identification of parasite species is very important as it permits the monitoring of drug resistance and parasite distribution according to climate changes. In this respect, molecular biology techniques can be used for parasite identification as well as for the control of infestation (Roeber *et al*. 2017).

Examples of the use of molecular biology techniques for parasite identification is the study of Barkway *et al*. (2011) that used LAMP technique to identify seven different *Eimeria* species in birds, an important protozoan in poultry production. The diagnosis is fast and inexpensive. Using tandem multiplex PCR, Roeber *et al*. (2017) obtained important results in the identification of nematode species that infest sheep, with the possibility to use any life cycle stage for DNA extraction (eggs, larvae, adult worms).

Molecular genetics and animal breeding

Animal breeding and genetics is a livestock production tool whose objective is to alter production rates by selecting genetically superior animals for reproduction. The genetic evaluation of sires for different traits of interest, as well as the economic weighting of traits, forms the basis for application to animal breeding. Breeding values are estimated based on pedigree information and collected phenotypes. The first major historical step in genetic evaluation was the introduction of artificial insemination. Artificial insemination permitted males to leave a large number of offspring, a fact that considerably increased the accuracy of breeding value prediction, in addition to facilitating the dissemination of superior genetic material.

The initial use of molecular genetics for animal breeding was very inefficient as studies of polymorphisms in candidate genes influencing economically important traits showed that the discovery of these genetic variations caused little or no alteration in the genetic evaluation itself (given some exceptions as described in item 2). However, the current paradigm of genomic selection, which is based on molecular biology techniques, has greatly changed the way genetic evaluation is done today.

Genomic selection is traditional selection combined with the use of SNP-type DNA markers spread across the genome. These markers increase the accuracy of breeding value prediction and permit the use of younger sires (due to the gain in accuracy), reducing the generation interval and increasing genetic gain (Abell *et al*. 2014; Dechow and Rogers 2018). The objective of this item is not to talk about genomic selection itself. This is the main line of study of the major research groups and breeding companies in the world and is much more related to the analysis of data for genetic evaluation than to the application of laboratory techniques, which is the objective of this review. However, it should be pointed out that large-scale genotyping of SNP was developed from practical laboratory knowledge and genomic selection would not exist without this knowledge.

In addition to genomic selection, many other studies can be conducted using SNP chips, including genome-wide association analysis that permits the identification of genes with large effects on the traits, linkage disequilibrium, copy number variations, identification of lethal genes, chromosome rearrangements, selection signatures, autozygosity, paternity testing, genetic variability, among others.

Genetic diversity

Molecular genetics also contributes to population studies designed to evaluate genetic diversity. Genetic diversity is important to maintain the variability of a population that is under the effect of domestication or selection and to initiate and maintain breed conservation programs. Molecular markers permit to evaluate the degree of heterozygosis of a population and thus to observe the possible effects of inbreeding. Population studies of inbreeding are then completed or, if no pedigree data are available for a population, inferences can be made in loco about its genetic variability (Kristensen *et al*. 2015).

The markers most commonly used to verify this variability are microsatellites, which are multi-allelic markers spread across the genome. Santos *et al*. (2016) report examples of the loss of genetic variability in tambaqui (*Colossoma macropomum*), a fish species of commercial interest, which is due to rearing in captivity and the lack of relationship control. Other genotyping methods such as SNP chips can be used for the evaluation of

genetic diversity. Makina *et al*. (2014) studied the population structure of six South African cattle breeds using an SNP chip. The authors found low to moderate genetic variability among breeds, as well as divergence between local breeds and those developed locally from European breeds, demonstrating the importance of conservation to cope with environmental changes. Mastrangelo *et al*. (2017) studying Barbaresca sheep, an endangered breed, showed the importance of use of SNP chips in small populations and future strategies that should be taken for better genetic conservation. Barbaresca sheep has a small effective number, high inbreeding and many loci under homozygosis. Genomic information has a crucial role for breed management.

Genome editing

Editing the genome is the latest molecular biology frontier reached in animal production. This procedure consists of the identification of a specify target and posterior mutation induction or gene knockout in an organism in order to achieve improvements in animal production that are positively reflected on the humans who depend on it. According to Petersen (2017), there are three main techniques of editing the genome. Their application to domestic animals is still in the field of research, but already shows results of potential applicability.

Examples of successful genome editing in livestock are the production of polled Holstein cattle (Carlson *et al*. 2016) by the introgression of a causal mutation in Celtic breeds through genome editing and interruption of the *MSTN* and *FGF5* genes in goats (Wang *et al*. 2015) to improve animal performance, as well as *MSTN* in cattle and sheep with an impressive phenotypic change (Proudfoot *et al*. 2015).

There are also applications of genome editing in domestic animals for biomedical purposes, such as the production of pigs in which the genes encoding cell surface proteins are deleted to reduce rejection of organs in xenotransplants (Butler *et al*. 2016).

It should be remembered that genome editing is not related to transgenics as in most cases it only induces DNA modifications in a species without combining it with the DNA of another species and may therefore be potentially more amenable in terms of legislation.

Conclusion

Molecular genetics has many applications in animal production. Knowledge of techniques and their applications is important for the management of intensive livestock production. The inclusion of disciplines or subjects in undergraduate and postgraduate courses that address the topics cited above is fundamental for the training of professionals to provide services to the market and to develop future technologies related to the area. This review was intended to gather the applications of these molecular techniques in livestock field.

Conflicts of interest

The author declares no conflicts of interest.

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