Animal biotechnology

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Introduction

BIOTECHNOLOGY provides the production of goods and services by using of living organisms or their parts. For thousands of years, several human activities, such as fermented foods production (bread, wine, yogurt, beer, etc.) are examples of the use of biotechnology. But it was only after the discovery of the DNA structure by Watson and Crick in 1953 and of Recombinant DNA technology in the 1970s that modern biotechnology has been designated for using of genetic information obtained directly from DNA.

In animal production, biotechnology can be used to increase food production, efficiency of production systems, quality of animal products and sustainability of the system. Examples of commercially available products generated through the use of biotechnological techniques are bovine growth hormone, used to increase milk production; recombinant vaccines for the prevention of diseases in cattle, pigs, sheep and poultry; genetic testing of DNA used in selection of animals with superior genotypes in breeding programs.

Given the breadth of this topic, the purpose of this text is to discuss the use of animal biotechnology through different techniques to analyze the information contained in DNA and application thereof in programs focused on the genetic improvement of species of livestock interest, such as cattle, pigs, poultry and others. In addition, this paper will discuss applications and prospects for the use of animal biotechnology, especially for cattle and poultry breeding.

Animal breeding

It was in the twentieth century that animal production shifted from a subsistence and extraction activity to a commercial activity. At that time, there was a growing demand for better performing animals, with greater adaptability to different environmental conditions. As a result, breeding programs for cattle, pigs, poultry, sheep, and goats among other animal species began to be developed. Using as a genetic basis the set of alleles of domesticated breeds, these programs were implemented intuitively, based on production and aesthetics traits of animals with superior phenotypes. Phenotype measurement cycles were established for the selection of animals with superior genotypes to be used as progenitors of future generations. Consequently, this selection process led to an increase in the frequency of favorable alleles that improved production traits.

Therefore, breeding programs have improved not only production levels

but also the adaptive potential of species of economic interest, without knowing the individual genes and molecular mechanisms associated with them. It should be noted that advances in the areas of statistics, through the development of more elaborate selection models, and computer science, with the development of increasingly sophisticated software, breeding programs have made significant progress.

Despite the gains observed in several traits (e.g., weight and milk, meat and egg production), the genetic potential estimation based exclusively on the animal's phenotype presents some limitations. How to predict, for example, the yield of carcass and parts and body fat without slaughtering the animal? And how to assess resistance to disease without pathogen challenge? These are some examples of traits that are difficult or expensive to be measured accurately in traditional breeding programs, thus limiting the increase in the improvement rates of these traits.

The G. Mendel's works, in 1865, established the laws of heredity by associating a hereditary component with observable traits. Ronald A. Fisher's study, in 1918, was the first to distinguish genetic variance from environmental variance and the partition of genetic variance into additive, dominance and epistasis variances. These studies enabled defining that the phenotype (phenotypic variance) could be broken down into two parts: one due to genetics (genetic variance) and another due to the environment (environmental variance). But it was only in 1944 that Oswald T. Avery demonstrated that the hereditary factor is determined by DNA (Coutinho et al., 2010).

Animal biotechnology

With the discovery of the DNA structure in 1953, and with Recombinant DNA technology coupled with the advent of molecular markers, both proposed in the 1970s, the possibility emerged for enhancing phenotype-derived information with DNA-derived information, with a view to making the process of identifying and selection of animals with superior genotypes more efficient. DNA sequence markers close to the region of interest (genes) would allow monitoring the segregation of alleles by generations and, therefore, the traits of interest associated therewith. These markers are identified by the association of segregating alleles with phenotypic values for the studied trait. Since the production capacity of an animal (phenotype) is the result of the interaction between the genetic material (genotype) and the environment, these markers would represent the possibility of animal selection based also on genotype, even before phenotype expression. The possibility therefore emerged for determining the genetic merit of an animal in the embryonic or young stage, without the need for an evaluation of production or progeny, which would lead to a faster and more effective selection.

Molecular markers therefore have been a powerful tool for identifying mutations that influence traits controlled by one gene or a few genes. However,

most of the traits of economic interest exhibit a polygenic inheritance pattern, and are determined by numerous genes of large and / or small individual effect and under the strong influence of environmental factors. The identification of alleles associated with these complex traits in the populations was only possible after the development and location of polymorphic molecular markers in the genome, particularly microsatellites and single nucleotide polymorphisms (SNP), allowing the construction of saturated genetic maps and, consequently, the mapping of quantitative trait loci (QTL). The integration of results from QTL mapping to those obtained by candidate gene strategies, studies related to gene expression involving quantitative PCR techniques, microarrays and sequencing may allow the dissection and understanding of the number, function, contribution and location of genes involved in the control of quantitative traits (Coutinho et al., 2010).

Identification of genes of livestock interest

The main goal of DNA analysis in domestic animals has been the dissection of the genetic architecture of traits of economic interest, determining the number of genes and the contribution of each of them to phenotype expression. This goal, however, has not been easily achieved, especially because the traits of interest present a quantitative nature, i.e., controlled by multiple genes, each contributing a portion to the phenotype.

Three main strategies have been used to identify genes of interest: QTL mapping, candidate genes and DNA and mRNA sequencing, including gene expression.

QTL

QTL mapping is based on the identification of chromosomal regions associated with the genetic variation of traits of economic interest. This identification is dependent both on the development of genetic maps saturated with polymorphic molecular markers and on a population structure that shows segregation for the trait of interest. The identification of microsatellite markers (Tautz, 1989) and SNP (Collins et al. 1998) has enabled the development of markers throughout the genome. Today, saturated genetic maps, along with the development of statistical methods, have enabled dissecting complex production traits, with the identification of chromosomal segments containing genes that determine muscle development and meat quality, for example.

The use of segregation analysis in informative families or experimental crosses for QTL mapping is well defined. A meta-analysis indicated twenty consensus regions in the genome of cattle, corresponding to regions containing QTL coincidence, mapped in independent populations for the same trait. Among these consensus regions, two were found to control milk yield, both located in chromosome 6, one at 49 cM and the other at 87 cM, explaining 4.2 percent and 3.6 percent of the genetic variance of this trait respectively (Khatkar

et al., 2004). The first of these regions (located near marker *BM143*) was associated with five milk production traits, namely protein yield, protein percentage, fat yield, fat percentage, as well as milk yield. For sheep, some results were obtained for milk production (Tascón-Diez et al., 2001; Barillet et al., 2005), growth and carcass traits (Walling et al., 2004) and resistance to parasites (Davies et al., 2006). QTL were also mapped for fleece trait in goats (Cano et al., 2007).

In cattle, CattleQTLdb[1] totaled 4,281 QTL mapped, with 98 associated with external traits, 316 with health, 907 with meat quality, 1,196 with milk production and 817 with reproduction. In poultry, 2,284 QTL were mapped for external traits (80), health (227), physiology (103) and production (1,874), according to ChickenQtLdb.[2] For pigs, PigQtLdb[3] of the 5,986 QTL mapped indicated that 377 were associated with external traits, 586 with health, 4,143 with meat quality, 607 with production and 273 with reproduction. Monsanto has over 4,000 autosomal SNP genotyped, of a total of approximately 6,000 pigs, to investigate the extent and range of linkage disequilibrium (LD) in the porcine genome (Du et al., 2,007), enabling associating traits of interest with DNA polymorphisms. For sheep, Walling et al. (2004) mapped QTL for body weight at eight weeks of age and muscle and fat thickness at 20 weeks of age, and McRae et al. (2005) identified QTL for body weight and fat thickness.

In Brazil, an agreement was established between Embrapa Swine and Poultry and ESALQ/USP for conducting studies on poultry genomics.[4] Thus, two reference populations have been developed for mapping QTL and genes associated with performance and carcass traits. These populations, in F2 scheme, were named CTCT and TCTC, as they originated from reciprocal layer (CC) x broiler (TT) crosses. For the TCTC population, linkage maps have already been constructed (Nones et al., 2005; Ambo et al., 2008) and QTL has been mapped for performance and carcass traits on chromosomes 1 (Nones, 2004; Nones et al., 2006); 2 and 4 (Baron, 2004); 3 and 5 (Ruy, 2004); 11 and 13 (Boschiero, 2006; Boschiero et al., 2009); 19, 23, 24, 26, 27 and 28 (Ambo, 2007); 9, 10, 12, 14, 15, 16, 17 and 18 (Campos, 2007; Campos et al., 2009a). A genome scan with 129 microsatellite markers has been completed for the TCTC population on 22 chromosomes, and the results have been published by Ambo et al. (2009) for performance traits; by Campos et al. (2009b) for fat deposition traits; and by Baron et al. (2010) for carcass traits. Boschiero (2009) fine-mapped a region of chromosome 1 of the TCTC population, and analyzed polymorphisms on *IGF1* and *JARID1A* genes, which are related to growth. For the CTCT population, the initial work involved the study of chromosomes 1, 3 and 4 (Rosario, 2007); the genotypic description of TCTC and CTCT populations (Rosario et al., 2009); and the construction of linkage maps (Rosário et al., 2010). Chromosomes 5 (Silva et al., 2009) and 2 (Guido et al., 2009) have also been studied for this population.

Other initiatives in Brazil are in pig genomics came from the Federal University of Viçosa and from Embrapa Dairy Cattle and Embrapa Livestock Southeast in bovine genomics. It is important to point out that these projects aim to map QTL for performance and carcass traits expressed under climate and breeding conditions in Brazil, thus facilitating the development of markers for the improvement of traits of national interest.

Although it enables identifying genomic regions containing genes of interest, the QTL mapping strategy has its limitations, one of which is the high cost, since it involves the development of an experimental population with at least two generations, which needs to be completely phenotyped and genotyped. Other limiting factors include the cost of genotyping for molecular markers covering the entire genome, the variable power of QTL detection, related to the informativeness of the alleles of molecular markers and QTL alleles, and also the heritability of the trait under study. Finally, a major constraint is the fact that the strategy indicates the chromosomal region that possibly contains the genes associated with the trait of interest, but the identified region may contain many genes. Moreover, this strategy defines neither the number nor the exact effects of these genes on the quantitative trait.

Nonetheless, QTL mapping was essential for the identification of genes responsible for traits of interest in domestic animals, including *diacilglicerol O-acetiltransferase* (*DGAT1*), which controls milk composition and production in cattle (Grisart et al., 2002), and *myostatin*, which controls muscle development in cattle (Grobet et al., 1997) and sheep (Clop et al., 2006).

Candidate genes

Another strategy of gene association with traits of economic interest in animal production is the study of candidate genes. These are genes of known biological action involved with the development or physiology of a trait of economic interest (Bryne & McMullen, 1996). Some successful examples of the use of this strategy are the *halothane* and *RN* genes, which are related to meat quality in pigs (De Vries et al., 1998), and the *myostatin* gene, associated with double-muscling in cattle (Grobet et al., 1997). A total of 13 SNP have been associated with four genes related to meat and carcass quality in cattle (Haegeman et al., 2005). The mutations identified in genes encoding calpain and calpastatin are now commercially used in animal selection for greater meat tenderness. Other examples can be found for cattle, pigs, poultry and sheep in Dekkers (2004).

The main limitation of this strategy is that only a small proportion of genes controlling quantitative traits are known. There are also difficulties in definitively establishing the effect of the candidate gene, since the identification of the causal variant for a gene of smaller effect might not be easily determined.

DNA and mRNA sequencing

The ability to identify genes by genome and messenger RNA (mRNA) se-

quencing determined by expressed sequence tags (EST) (Adams et al., 1991; detailed by Hatey et al., 1998) promises to overcome some of the technical limitations of previous strategies. Genomic sequence information contributes to understanding how genetic variation influences the trait of interest by enabling mapping this trait at a precise location in the genome (Schmutz & Grimwood, 2004). Saturated genetic maps containing numerous microsatellite markers and SNP, and haplotype maps containing mapped QTL will have direct correspondence with the genome sequence in commercial and experimental populations. Candidate genes may be identified directly in the body and in the target tissue, and re-sequencing of QTL intervals for the identification of influencing causal mutation(s) will also be facilitated.

EST collections are being established for tissues of economic interest in several domestic species. The chicken database, for example, has more than 599,000 EST (reviewed by Fadiel et al., 2005). The EST collection obtained for cattle (*Bos taurus*), deposited with NCBI (dbEST), now totals over 1,315,093 and more than 641,896 for pigs (*Sus scrofa*).

In addition to obtain the gene's sequence and position in the genome, these collections of DNA and mRNA sequences are also the basis for the construction of microarray platforms, which are developed for analyzing the gene expression pattern in large scale. This strategy is based on the hypothesis that organisms with different phenotypic traits exhibit differential expression of genes related to these traits. The overview of gene expression enables understanding temporal and spatial changes in gene activity during cell development and differentiation, which contributes to the identification of specific or differential gene expression in different breeds or strains. These arrays therefore are essential tools for determining the biological functions of genes by the tissue-specific expression pattern and thus for supplementing the biological information obtained by QTL mapping and sequencing projects, in order to promote the identification of genes associated with complex traits, such as those of economic interest (Andersson & George, 2004).

This strategy, however, also has its limitations. Microarrays require prior knowledge of the gene sequence spotted onto the platform, which limits the availability of arrays for all species of interest. Cross-hybridization between transcripts and target sequences (caused by gene duplication) and limited sensitivity of EST arrays for transcripts that are infrequent, poorly represented in libraries and usually important regulatory genes, are also limiting factors in this strategy. More importantly, the arrays help identify metabolic pathways associated with the trait of interest, but not the responsible gene.

An advantage of genome sequencing is the large-scale identification of SNP. These have allowed the development of novel methodologies and strategies for mapping genes of interest, since they are extremely useful markers to promote fine mapping, whose goal is to define the smallest genomic region containing a QTL (Carlson et al., 2004).

The chicken was the first domestic animal to have its genome sequence published (Hillier et al., 2004). The sequence of the 1.05 Gbp genome was obtained from DNA from a single female *Gallus gallus* (Red Jungle Fowl), the ancestor of the domestic chicken. The physical map of the bovine genome was published in 2007, with the contribution of more than 20 research groups from eight countries (Australia, Brazil, Canada, Scotland, U.S., France, Italy and New Zealand) over a period of five years. This genetic map consisted of a large database containing 422,000 DNA sequences and more than 17,000 markers.[5] Finally, the sequence of the bovine genome from a female Hereford was published by Elsik et al. (2009).

In chickens, over 2.8 million SNP have been identified by comparing the sequence of the ancestor's (*Gallus gallus*) genome with sequences obtained for three domesticated breeds: a male from a broiler chicken (White Cornish), a female from a layer chicken (White Leghorn) and another female from an ornamental species (Chinese Silkie) (Wong et al., 2004). Groenen et al. (2009) published the first linkage map with 8,599 SNP. In cattle, 35,000 SNP markers have been identified and validated, which enabled the construction of the bovine haplotype map (*bovine hapmap*) (Gibbs et al., 2009). More recently, panels of 50,000 and 770,000 SNP have been made commercially available.

Thus, important advances have been achieved in animal genotyping using SNP chips with platforms already available for the simultaneous detection of thousands of SNP. These SNP chips should promote a revolution in animal genomics, by allowing the scanning of the genome of an experimental population for thousands of SNP simultaneously, at lower costs and more quickly as compared to what is being done today with the use of microsatellites. Thus, it will be possible to make use of the so-called genomic selection.

Genomic selection

According to Grattapaglia (2010), the genomic selection strategy involves the simultaneous selection for thousands of markers, so that the majority of genes or genomic regions involved in the control of multiple quantitative traits will be in linkage disequilibrium with one or more genotyped markers. This marker-based approach has some advantages: it does not require prior discovery of QTL, has a highly selective accuracy, avoids biased estimates of the effects of genes and/or individual QTL, captures variation due to small effect loci, efficiently contemplates low heritability traits, and enables the application of prediction models to all families in the breeding program.

An example of the use of genomic selection was announced in January 2009 by the U.S. Department of Agriculture (USDA), which performed the genomic evaluation of Holstein bulls. Initially, 5,800 proven bulls from artificial insemination centers were genotyped, of which 4,422 were Holstein bulls, 1,149 Jersey bulls, and 228 Brown Swiss bulls. Currently, the number of genotyped animals exceeds 20,000. All bulls were genotyped using the Illumina Bo-

vine SNP50 BeadChip. This test enabled genotyping bulls for 58,000 molecular markers, of which 39,835 were related to economic traits.[6]

But how does this work in practice? Until the last summary of 2008, young bulls exhibited between 35 percent and 40 percent confiability for production traits based on pedigree alone. Since January 2009, young bulls have shown an increase in proof confiability standards, thanks to genomic evaluations. These evaluations increased the confiability of production traits to 65-70 percent. Confiability for type traits increased from 30-35 percent to 60-65 percent and health traits from 25-30 percent to 55-60 percent. So, with genomic evaluations, the confiability the proof of these young bulls increased twice in relation to the previous confiability, when genetic evaluation was based solely on phenotype.[7]

Another example was announced by the CRV group, which heads the CRV Lagoa in Brazil, a genetic center headquartered in Sertãozinho (SP). This company will introduce several changes in the selection of young bulls participating in the Insire program, including increasing their selection from the current 1,300 to 2,600 animals starting September 1st, 2010, through the inclusion of information obtained directly from DNA in its breeding program.[8]

Applications of animal biotechnology in breeding

Genomics has contributed to the advancement of animal biotechnology since the 1990s. The most striking example of this contribution was demonstrated with the *halothane* gene in pigs, which is associated with increased muscle deposition in the carcass, but with a greater propensity to produce PSE (pale, soft and exudative) meat (De Vries et al., 1998; Bridi et al., 2006). In the past, the test for identifying animals free of this syndrome was performed with halothane anesthesia, using a laborious method that did not enable distinguishing normal homozygous animals from those carrying the mutation in the population. The identification of the causative mutation of this condition led to the development of a simple genetic test that enables, from a sample of the animal's fleece, identifying normal, heterozygous and recessive individuals, thus facilitating the establishment of strains free of this mutation (Fujii et al., 1991). Today, virtually all pigs in breeding programs are tested for this mutation.

Another example was the identification of a mutation in the *myostatin* gene responsible for the greater muscle development trait (double muscling) in beef cattle breeds Belgian Blue and Piedmontese. The phenotype's genetic basis was explored, leading to the identification of several mutations in the *myostatin* gene (McPherron & Lee, 1997; Grobet et al., 1997). These mutations result in non-functional proteins that lead to a significant increase in the animal's muscle mass (~ 30%), birth weight and nutritional efficiency, and this increased muscle mass comes from hyperplasia effect (increased number of fibers) especially. However, this phenotype also causes impairments related to the decreased amount of intramuscular fat (marbling), female fertility and stress tolerance (Potts et al.,

2003), and therefore there is no interest in fixing these alleles in certain breeds. Molecular markers have been useful in the direct identification of this mutation by genotyping the population. Obtaining these genotypes beforehand helps to guide mating and embryo transfer within the herd available for genetic breeding.

Examples in other species include: DNA markers in gene *ESR* (*estrogen receptor*) in pigs were associated with the number of live-born piglets and total live-born piglets in the first and last parities (Alfonso, 2005). In sheep, in the Booroola Merino breed, the *BMPR-IB* gene was related to fertility, and in the Invedale and Hanna breeds the *BMP15* gene was associated with ovulation (Liu et al., 2003). Additional examples can be found in Dekkers (2004).

Contributions to the evaluation of meat quality in beef cattle by mutations in the genes that determine marbling and tenderness traits have been published. Tenderness is determined by the action of calpain-calpastatin complex enzymes, which act on the muscle after slaughter (Koohmaraie et al., 1995). Calpains contribute to meat tenderness because they are intracellular endopeptidases capable of degrading proteins of the myofibrils that make up the muscle fiber (Wheeler & Koohmaraie, 1994). The activity of calpains is inhibited by the action of calpastatins, which therefore have a tenderness repression role (Pringle et al., 1997). Marbling, in turn, results from intramuscular fat deposition, promoting meat flavor and succulence. Polymorphisms in genes encoding m-calpain (Page et al. 2002; White et al., 2005), lysyl oxidase and calpastatin (Barendse, 2001; Drinkwater et al., 2006) have been detected and associated with tenderness, especially in *Bos taurus* breeds (Barendse, 2001, 2003; Barendse et al., 2004). For marbling, associated genes have been those encoding leptin (Buchanan et al., 2002) DGAT1 (Thaller et al., 2003), TG (Barendse et al., 2004), RORC (Barendse, 2003), GH1 (Schlee et al., 1994), SCD, mitochondria (Mannen et al., 1998, 2003), mitochondrial transcription factor A (Jiang et al., 2005) and FABP4 (Michal et al., 2006).

Although several results related to genomics in beef cattle are still experimental, many commercial DNA tests have been available on the market to assess meat quality (Hocquette et al., 2007). As an example, we have genetic tests for tenderness conducted by private companies such as the IGENITY TenderGENE® test (Merial Ltd., Atlanta, GA) and GeneSTAR Quality & Tenderness® (Genetic Solutions Pty. Ltd. Albion, Australia). However, many of these tests are being used in independent studies to confirm the association of SNP polymorphisms with marbling and tenderness traits (Page et al., 2004; Casas et al., 2006; Morris et al., 2006; Rincker et al., 2006), but not all have been validated, making the efforts to expand the frontiers of genomics even harder.

A major challenge in applying some of the polymorphisms initially associated with meat tenderness was the transfer of information originally obtained in *Bos taurus* to *Bos indicus* cattle, since these animals are known to have lower

meat tenderness compared to taurine cattle. Polymorphisms identified in the *μ-calpain* gene (*CAPN1*), for example, exhibited diversity in *Bos taurus* genotypes, but not in *Bos indicus* (Page et al., 2002, 2004; Casas et al., 2005). There are a number of SNP within or very close to this gene and two of them seem to be informative in *Bos indicus* and *Bos taurus* (position 316 and 4753 bp), but a SNP at 530 bp seems to be informative only in *Bos taurus*.

Marbling is a more difficult trait to work on than tenderness, since its evaluation is made by visual inspection of the carcass, and therefore it is a subjective measurement with a significantly higher associated error compared to tenderness. This requires large samples of animals for the average to be appropriately estimated. Studies evaluating less than 1,000 animals do not enable confirming an association between DNA and tenderness markers (Hocquette et al., 2007). It should be noted that a partnership between the School of Animal Science and Food Engineering of the University of São Paulo and the Merial® company has evaluated several molecular markers in *Bos indicus* cattle in Brazil. These markers have been previously associated with marbling and tenderness trait in *Bos taurus*. Another Brazilian example was provided by the Beef Quality project coordinated by several units of Embrapa and Brazilian universities.

Many efforts have been made to increase the reproductive efficiency of the Brazilian bovine herd, which is characterized by low pregnancy rates and high rates of embryonic mortality. Reproductive traits allow early selection of animals, enabling greater selection intensity and hence greater efficiency of the productive system. In Nelore cattle, for example, calves are born to cows between 2.5-3.0 years of age, which is a disadvantage compared to the 2.0 years of age in European breeds. In Brazil there are animals with genetic ability, but which are affected by different feeding and management environmental conditions, thus hindering selection. It is difficult to conduct an accurate genetic evaluation of many reproduction traits. Besides, heritability traits associated with fertility are considered low (between 2 and 15 percent), although recent studies in Nelore cattle have shown high heritability for sexual precocity (Silva et al., 2005).

Thus, two strategies have been used in an attempt to associate molecular markers with fertility traits: QTL mapping and candidate genes. QTL have been mapped for fertility traits, including: ovulation and multiple ovulation rate (Kappes et al., 2000) and twinning rate (Lien et al., 2000). The genes encoding the gonadotropins-releasing hormone (Schneider et al., 2006), leptin (Liefers et al., 2005), and the bovine luteinizing hormone receptor (Hastings et al., 2006) have been investigated as candidate genes for fertility traits. The *leptin* gene has been associated with reproduction-related traits because it encodes a hormone that participates in the regulation of energy metabolism, food consumption behavior and reproduction in many animals, in addition to participating in other events, including puberty. It is considered a candidate gene due to its known relationship with adipose tissue mass in the body and puberty. Leptin levels in

the blood are related to the animal's body fat levels: malnourished prepubescents do not enter puberty until they are properly nourished; likewise, cycling cows stop cycling when facing extreme periods of malnutrition. Therefore, the protein encoded by the *leptin* gene acts as a factor in the reproduction system. Polymorphisms in this gene could influence the regulation of metabolism and affect weight gain, and these mutations could be used in breeding programs. Some mutations in this gene have been identified in cattle (Buchanan et al., 2002; Choudhary et al., 2005), and gene expression studies have also showed an association between the high expression of this gene with the low expression of the *NPY receptor* and the activation of precocious puberty in *Bos indicus* heifers (Vaiciunas et al., 2008). However, to date no molecular marker has been developed to allow selection by genotyping with a view to sexual precocity.

Other candidate genes associated with fertility have been investigated for functional genomics focusing on oocyte maturation (Dalbies-tran & Mermillod, 2003; Vallee et al., 2005; Massicotte et al., 2006), regression of the corpus luteum (Casey et al., 2005; Bønsdorff et al., 2003), bovine oviduct epithelial cell function (Bauersachs et al., 2003, 2004), endometrium during the estrous cycle (Bauersachs et al., 2005), and development of preimplanted embryo (El-Halawany et al., 2004; Sirard et al., 2005). The expected result of this strategy is the identification of genes or gene pathways which, at the appropriate time and location, regulate female fertility.

Another important contribution in cattle is resistance to ecto- and endoparasites. The clamor for reducing the use of chemicals to combat these parasites has been widely publicized, with a view to reducing the chance of contamination of both meat and meat products and the environment. Furthermore, the systematic use of these products can make the parasite resistant to active principles.

Suggestive QTL were mapped for tick [*Riphicephalus (Boophilus) microplus]* resistance on chromosomes 5, 7 and 14 in a population consisting of F2 from the *Bos taurus* (Holstein) x *Bos indicus* (Gir) cross (Gasparin et al., 2007), and the expression of genes involved in the immune response to gastrointestinal endoparasites (*Cooperia, Haemonchus and Oesophagostomum*) by means of quantitative PCR was conducted in *Bos indicus* (Nelore cattle) by Bricarello et al. (2008). These studies aim to identify the genes controlling resistance to ecto- and endoparasites, enabling the selection of cattle with greater genetic resistance. Probably, this will reduce the use of drugs and chemicals against parasites, in favor of healthier, chemical-free meat, thus reducing sanitary barriers imposed by international trade in beef.

Research has been conducted in order to refine the location of a QTL previously identified by fine-mapping in poultry. Kim et al. (2006) used eight additional microsatellite markers located on GGA1 to fine-map a sample of 314 F2 chickens from a cross between two commercial broiler lines with different susceptibility to coccidiosis. The researchers detected the association of marker

LEI0101 with disease resistance (LOD> 2.7) and also between markers *LEI0071* and *LEI0101* (at 254 cM) when analyzing four rather than twelve families (LOD = 3.74).

In a region of 50.8 cM (*LEI0079-ROS0025*) on GGA1, in which QTL were mapped for live weight, carcass weight and fat traits, nine microsatellites were added and an analysis was conducted for an additional eight families of an F2 population from a cross between a broiler breed selected against abdominal fatness and a layer breed native to China, totaling twelve half-sib families (1,011 animals) and twelve markers (Liu et al., 2008). The previously mapped QTL were confirmed, some with shorter confidence intervals. From previous studies in which 15 QTL associated with resistance to Marek's disease in chickens were mapped, Heifetz et al. (2009) used six generations of a population derived from the cross between two commercial strains of White Leghorn chickens (total of 1,615 individuals). A total of 217 microsatellite markers and 15 SNP were used, identifying 21 QTL regions associated with resistance to Marek's disease on different chromosomes (GGA1, 2, 3, 4, 5, 8, 9, 15, 18, 26, and Z). In the regions in which QTL were mapped, the confidence intervals were twice shorter compared with the previous study.

Based on the candidate gene strategy, Ledur et al. (2007) compiled several studies associating polymorphisms in candidate genes with traits of economic interest. According to these authors, growth traits were associated with polymorphisms in the *ghrelin* (Fang et al., 2007), *lambr1* (Huang et al., 2007), *growth hormone* and *growth hormone receptor* (Feng et al., 1997), *MC3R* (Jiang et al., 2002), *MC4R* (Qiu et al., 2006), *IGF-II* (Yan et al., 2002), *TGF-* (Li et al., 2002), *myostatin* genes (Gu et al., 2002; Ye et al., 2007); abdominal fatness was associated with polymorphisms in the *growth hormone* and *growth hormone receptor* gene (Feng et al., 1997) as well as in the *UCP* (Zhao et al., 2002) and *PPARA* genes (Meng et al., 2002); residual feed intake was associated with polymorphisms in the *PEPCK-C* gene (Parsanejad et al., 2003). Wu et al. (2006) showed that abdominal fat weight at 12 weeks in chickens with genotype GG was 34.26 and 28.71 percent greater than in chickens with genotypes AA and AG respectively, indicating that the *Asp216Asn* polymorphism in the *PPARGC1A* gene could be used as a new potential molecular marker for selection against abdominal fatness, without interfering with improvement in growth rate. Zheng et al. (2009) found higher levels of *FGFR2* expression in laying hens as compared broilers, although no significant difference was found in *FGF2* expression.

Other examples were provided by Zhang et al. (2009), who identified two SNP in the *calpain* 3 gene in chickens. Haplotypes were built, and then the association between SNP genotypes, haplotypes and diplotypes with the traits evaluated was analyzed. Several associations of genotypes and haplotypes and diplotypes with live weight, carcass weight, breast muscle weight and thigh mus-

cle weight were found. Nie et al. (2005) studied polymorphisms of the *growth hormone* gene in chickens and found associations with body weight, weight gain and length of shin bone. In our group, Souza (2004) studied polymorphisms in five genes related to muscle development (*MyoD*, *Myf5*, *myogenin, MRF4* and *myostatin*) in parental strains of the Embrapa population, and found associations of the *myogenin* gene with live weight at 42 days, weight gain, and weight of carcass, wings, abdominal fatness, liver and lungs. Ninov et al. (2008) investigated polymorphisms of the *leptin receptor* gene in the same population and found six polymorphisms, two of them in the TT line (broiler) and four more frequently in the CC line (layer). Two SNP of the *leptin receptor* gene were associated with various traits. The *C352T* SNP was associated with crude protein and ash, and liver, chest and carcass yield. The *G915A* SNP was associated with feed intake, and yield of lungs and thighs and drumsticks.

Nie et al. (2005) studied the polymorphisms of the *growth hormone* gene in chickens and found associations between body weight and weight gain with the candidate gene studied. Wang et al. (2005) studied the *IGF2* gene in chickens and identified a polymorphism associated with abdominal fat weight, birth weight and breast weight. Zhou et al. (2005) identified polymorphisms in candidate gene *IGF1* associated with growth, metabolic traits, body composition and bone integrity. A polymorphism in the region of *IGF1* gene promoter was associated with most of the traits studied.

Gene expression analysis by microarray has also contributed to animal breeding. This technique enables determining the differential expression of thousands of genes in a single experiment. Some results of the use of this strategy can already be found for the identification of candidate genes associated with meat quality in cattle. For example, a microarray containing 9,274 transcripts expressed in muscle and adipose tissues of cattle has been developed (Lehnert et al., 2004) and used to identify differences in the gene expression profiling of muscle tissue in Brahman breed during nutritional restriction (Reverter et al., 2003; Byrne et al., 2005); to study the mechanisms involved in *in vitro* adipogenesis in fibroblasts in differentiation (Tan et al., 2006); and to investigate differential gene expression between Black Japanese and Dutch cattle (Wang et al. 2005). A microarray containing 5,500 transcripts identified in the muscles of three days old pig-fetuses was also constructed to detect differences in the expression profiling between *psoas* (red muscle) and *Longissimus dorsi* (white muscle) (Bai et al., 2003). In chickens, Cogburn et al. (2003) identified differential gene expressions in liver of strains subjected to divergent selection for weight gain, while studies conducted by Bourneuf et al. (2006) led to the identification of genes expressed in the liver and associated with fat deposition. Also for chickens, a microarray was constructed from 32,773 transcripts obtained from multiple tissues and at different development stages, corresponding to 28,000 chicken genes (Burnside et al., 2005[9]), which can be used to identify genes associated with chicken white meat quality.

Another microarray was constructed from 6,887 transcripts associated with innate immune response, and used to characterize expression patterns of genes involved in disease control both in cattle and sheep (Donaldson et al., 2005). A total of 45,383 genes identified in the mammary gland and digestive system of bovines has also resulted in the construction of a microarray, which was used for the identification of estrogen-responsive genes (Li et al., 2006). Using a microarray of bovine oligonucleotide, Ollier et al. (2007) identified 161 differential expression genes in the mammary gland involved in the metabolism of milk protein, lactose and lipids, in a group of goats with food deprivation and another group with no nutritional restrictions. In sheep, Diez-Tascón et al. (2001) detected differences in the expression of nematode-resistance genes by microarrays containing 10,204 bovine genes. Fahrenkrug (2007) reported the construction of 20,400 swine protein-annotated oligonucleotides-microarray.

Given the above, various strategies have led to a better understanding of the complex biological system involved from conception to the final phenotype expression of livestock.

Perspectives

Animal biotechnology with the use of molecular markers has allowed programs for the genetic improvement of commercial production animals to benefit from existing technologies. Thus, new sources of molecular information have been established for QTL mapping, including genome sequencing, which will further facilitate the identification of specific favorable alleles for breeding. Associated with this scenario, genomic selection is also a reality, since SNP panels have been developed to promote large-scale genotyping. The company Illumina already offers a panel with 800,000 SNP for cattle and 60,000 SNP for chicken.

The rapid progress in the discovery of genetic variation patterns capable of predicting animal performance can therefore be expected as a result of international and national efforts. SNP panels should promote a revolution in animal genomics by allowing genome scan for thousands of SNP simultaneously, at a lower cost and more quickly compared to what is being done today using microsatellites.

It will therefore be possible to include not only the information about one or a few SNP in predicting the genetic value of each animal, but also of thousands of SNP associated with different traits of interest for breeding, due to their degree of genetic correlation. This is a broader view of the complex biological system involved in the establishment of phenotypic patterns such as increased meat, milk and egg production among others.

Therefore, associated with genomic selection, the tendency will be to apply the systems biology strategy to animal breeding. Systems biology emerged from the successful outcomes of genome strategies, as a result of animal biotechnology, and aims to develop statistical and analytical methods capable of combining the information obtained from genomics, computational biology, chemistry and mass spectrometry, among others.

Finally, genome selection and systems biology associated with classical strategies for improving phenotype and genotype selection should result in a more comprehensive application of tools for the analysis of DNA, RNA and proteins in animal breeding, in the quest for greater efficiency in animal production systems.

Notes

1 Available at: <http://www.animalgenome.org/QTLdb/cattle.html>. Access on: 21 set. 2010.

2 Available at: <http://www.animalgenome.org/QTLdb/chicken.html>. Access on: 21 set. 2010.

3 Available at: <http://www.animalgenome.org/QTLdb/pig.html>. Access on: 21 set. 2010.

4 Available at: <http://www.cnpsa.embrapa.br/genomafrango/genomafrango.html>.

5 Available at: <http://www.bovinegenome.org>.

6 Available at: <http://www.semeia.com.br/site/artigo.php?ID=115&IDC=3>.

7 Available at: <http://www.semeia.com.br>.

8 Available at: <http://www.gazetadigital.com.br>.

9 Available at: <http://www.affymetrix.com/products/arrays/specific/chicken.affx>.

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ABSTRACT – Animal biotechnology is providing new tools for animal breeding and genetics and thus contributing to advances in production efficiency and quality of animal products. However, the progress is slower than anticipated, mainly because of the difficulty involved in identifying genes that control phenotypic traits of importance to the animal industry. Three main strategies: QTL mapping, candidate genes and DNA and mRNA sequencing have been used to identify genes of economic interest to animal breeding and each has advantages and disadvantages. QTL mapping allows identification of the genomic region that contains the genes, but the confidence interval of the regions is usually large and may contain several genes. Candidate gene approach is limited to our restricted knowledge of the biological function of the genes. Sequencing of genomes and expressed sequences tags can enable identifying gene position and metabolic pathways associated with phenotypic trait. Integrating these strategies using bioinformatics software will allow identifying novel genes for animal production. Then, animal breeding programs will include the information from DNA directly on evaluation of genetic value of livestock production.

Keywords: Poultry, Bovine, Genomics, Molecular marker, Animal breeding, Swine

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