Biotechnology in Animal Husbandry 22 (1-2), p 9-16, 2006 ISSN 1450-9156
Publisher: Institute for Animal Husbandry, Belgrade-Zemun UDC 636.

ADVANCED BIOTECHNOLOGIES IN ANIMAL PRODUCTION AND REPRODUCTION[1]

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The biotechnological applications in zootechnics are and will be more and more a reality in the economic growing strategy of a country, because they answer both commercial and socioeconomic need (*Madan, 2005*). The biotechnology has been practiced in the food-producing since the beginning of human history. In some way the different evolution of the various breeds, since the beginning of the domestication of animal species, could be seen as a biotechnological process carry out or 11.000 years, on the basis of nutritional, social and environmental need, with only a relative understanding of the involved molecular process (*Wheeler et al., 1993*).

During this period, at an increasing understanding of the process, involved with the determining factor of the desirable traits, we have had a better control of these traits. In the last century, since the rediscovery of *Mendel's work (1866*), the science have localized in the chromosomes the hereditary factors and then have put the basis of the molecular genetic, which have clarified the transcription and translation of DNA in protein and the regulation of this processes; in the same time the development of statistic study of the populations' quantitative traits was used, in food producing, to increase the appearance and conservation of desirable traits. In only 20 years the fast progress of the recombinant DNA technology, through embryo technology and genetic manipulation allowed to control in a ever and ever direct way many trait of different interest. The present biotechnology's state permit to manipulate the molecular factor at a level that arise ethical question in different camp (*Kaiser, 2005; Einsiedel, 2005*), also in the scientific community and that forced the public opinion, ever difficult to inform about so complex subject, to require to the legislative agency the creation of barrier for experimentation and a major safeguard.

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[1] Review paper – Revijalni rad
Presented as invited paper the 8th International Symposium «Modern Trends in Livestock Production», Belgrade, October 5th to 8th, 2005 – Uvodno predavanje na 8. međunarodnom simpozijumu «Savremeni trendovi u stočarstvu», Beograd, 5.-8. oktobar, 2005.

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In the same time the world of zootechnics undergo a series of deep social and productive change: the general reduction in all the occidental world of the soil use as food farming involve a decrease of the available animals, incrementing the breeder's need of technologies able to maintain and increase the animal production, according to the market's demand. The need of technologies is not limited to the quantitative subject but also qualitative, because the opening to a market more and more globalized require both a major standardization of the qualitative level with the request of methods more sensible and reliable, and the need to safeguard from unavoidable sophistication particular production of excellence.

In general the rapid biotechnologies' evolution of the last 20 years and in particular of the several methods based on DNA, have made their application in zootechnics, for both the potentiality of molecular manipulation and the remarkable inquiry's sensibility, a valid answer to the several needs, required to the modern animal production by the society and the market.

The biotechnologies in zootechnics, thought initially to be used in the production of vaccine and diagnostic's kit, have became a powerful tool to improve the animal production at different levels.

At level of livestock's genetic improvement the present increase in the molecular markers' use for trait of particular interest or of excellence in the selection of germ-plasm, associated to different techniques of in vitro fecundation, embryos' manipulation and their transfer, have allowed to obtain in a short time an animals' population in which the genetic aims are maximized and to better preserve typical breed linked to excellence products.

At this topic belong the experiments of cloning, aimed to improve the techniques of embryo's manipulation and to clarify complex physiological process, related to different research area: from the enhance of the reproductive yield to the study of aging molecular factor.

A second level, still experimental, concern the modification of the food products with the introduction of probiotic and protective factors, produced directly by the animal: not only an increment of the quantities, but also a qualitative and nutritional enrichment, obtained by the overespression of genes already present or by introducing in the germinal line, with the successive expression of exogenous genes; also this results are maximized by techniques that provide the increase and the use of modified embryo in the first development's stage.

A third level is the traceability, which in the agro-food area, have became an indispensable to evaluate transparency within the food's productive chain. The possibility to follow the product into the weaving fac-

tory, implementing, as parameter, particular molecular markers, linked to the animal in such way that we can correlate/bring back the product to the animal is actually a desirable aim in short time.

In conclusion the union between biotechnology and zootechnics is bound to increase during the next decade, because at the same time with the improvement of molecular knowledge involved in important parameters for the zoo technical market, such as the yield, there will be an increase in the ability to manipulate these parameters at molecular level, strengthened the interested traits for food production. Besides the biotechnology will offer the possibility to modify the different population, to enrich the qualitative content of the products. Obviously all that will require from the legislative agency a regulation, consistent with the need of the public opinion and of the market. Indeed the biotechnological applications to the zootechnics, like in other area in which are implemented, will not be separated by bioethics valuation.

Biotechnology applied to the genetic improvement of livestock

Traditionally genetic improvement was ever aimed to emphasize the traits that increase the livestock's survival in a generally adverse environment, this process permitted the development of phenotypic traits which defined the different breeds in the animal species used in the productive process. The creation of these phenotypic pools allow a controlled selection of the more interesting character for the market: such as the growing yield, fertility, composition of the derived food products, and pathology resistance. The biotechnologies allow several level of manipulation during the animal's growth *(Mapletoft et al., 2005*); actually are mainly used in Buffalo, cattle, horse, swine and minus prominently in goat.

A first level is the choose and selection of the traits that will be emphasized: in general the interest traits can be linked to a single gene, but more often are the expression's result of the interaction of more genes which control is more difficult. (*Williams, 2005*) such as the QTL. Biotechnologies have introduced the use of molecular markers based on polymorphism of DNA to characterize the interest traits. The implemented techniques are based on DNA fingerprinting: RFLP and AFLP. DNA extracted from the animal is digested by restriction enzymes and the risen fragment is separated on different kind of electrophoresis gel, defining typical patterns of the animal based on different fragment's migration; the selection of the searched polymorphism can be with several methods: hybridization through probe for the interest markers (RFLP) or through an

amplification of the fragment ligated to end-specific adaptor molecules, which base are chosen by the user, then the products are amplified with a second selective PCR (AFLP) and defined in a electrophoresis pattern this techniques is more sensible than RFLP and permit to detects more polymorphisms. At this level is strategical the choose of the kind of marker to use (SNPs, microsatellites, polymorphism of different known locus). Future approaches could be developed, with the increase of the knowledge, thanks to the sequencing project for the several zoo technical species and to the setting up of further genomic banks; while the implementing of new techniques, now applied in biomedical area (array) will lead up to a better analysis of the desired traits, in the genetic selection.

A second level is the reproductive biotechnology (*Basrur et al., 2005*), which in the last 60 years evolved very shortly: starting with the development of artificial insemination's techniques, allowed by the cryoconservation of first the sperm and then the oocytes, and getting to initially transfer and then manipulate the embryos by in vitro insemination's techniques and embryo analysis. The possibility to safeguard from infection or other contamination in a first time the germ plasm and then the zygotes and embryos has been of primary importance to improve the fertility in cattle and have allowed to implement a series of methods for the genetic selection, in particular in this subject is very important for the zoo technical implications the possibility to determine the sex through flow cytometric of sperm and recently using PCR (*Mapletoft et al., 2005)*. The possibility to split the embryos first of the implant to increase the fertility, united to cloning techniques can improve the knowledge of the metabolic and physiological processes, linked to the animals' development and then in a long term at their survival.

Transgenic: food product's qualitative improving

Transgenic, applied to zootechnic, determine not only a series of productive effects but also ethical and legislative problems of difficult resolution.

A first effect on the food production is the reduction in a few generations of the required time to improve a series of traits, linked generally to quantitative genes, such as the quali-quantitative products' composition (*Wheeler, 2003; Murray, 1999*), disease resistance and the growing yield.

But transgenic allow as principal aim, the exogenous genes expression in other species, enlarging the mean of animal production and introducing new product; the most relevant effect is the creation of new

breeds characterized by exogenous trait, in case of modification of the germinal line.

The used methods (*Faber et al., 2003*) are very different in function of the aims which are researched, of the interested species, in fact same species (cattle) are very difficult to transform; actually a preferential techniques not exist but there are a series of these (*Niemann et al., 2005; Wheeler et al., 2003*): from pronuclear microinjection, the most used, to several others such as somatic nuclear transfer, lentivirus injection and sperm-mediated gene transfer method. All these present different problems of integration, localization and expression of interest's transgene.

The solution seem be the combination of cloning and transgenic techniques which should lead to an increase of the efficiency of the process of integration and to a decrease of the costs in the long period. Actually the great limit of transgenic is the sparse knowledge of the physiological effects linked to the genetic modifications. Moreover in zoo technical area the implementation of these methods is more difficult for the lack of completed sequencing project (cattle) and for the high costs that allow their use only in research centers.

Traceability: a biotechnological approach

Biotechnologies allow to detect different kind of molecular markers, for their sensibility, in different areas are used the DNA based markers, which can be implemented not only to select, as described before, interest's trait but also to follow the animal product, through the weaving factory, within the technological transformation's processes, defining its traceability (*Lupien, 2005; Popping, 2002*). At present the weaving factory's food traceability is represented only by a documental production, which not safeguard completely from errors and frauds. In the same time the tutelage's need of the consumer in animal production is a priority stated by society (*McKean, 2001)* and institutions, which promote researches that develop methods to join scientific data to the actual documentation. For this in the scientific and zoo technical world the concept of a "identity card of the product" which show a series of scientific parameter, that allowed a fast traceability of the product by the client, is becoming more and more important. The implementing of DNA based markers as traceability parameters (*Loftus, 2005; Vazquez et al., 2004; Rodriguez et al., 2003*), in a long term, could substitute the actual documentation.

The principal techniques implemented is the PCR, united to the pattern's analysis of amplified DNA on electrophoresis gel, so that the typical band's pattern would be recognizable through the weaving factory

up to the original animal's product. The principal problem is the choose of markers that are different in function of the considered final product's composition.

Total DNA's composition in an alimental matrix is generally a mix of animal, microbial and vegetal origin, with different percentage of this three parts in function of its characteristics and of the productive processing. The chosen markers should be specific, easy detectable and resistant to the technological processing. In particular the specificity is given by markers' polymorphism: higher is polymorphism more sensible will be the analysis.

The implementing of techniques used in biomedical area (array), which actually present excessive costs to an extensive use in zootechnics, an improve in the choose of DNA markers, thanks to completion of the several sequencing project, a better understanding of the molecular process in the manufacturing environment through weaving factory will guarantee a traceability based on molecular technique.

In conclusion the implementing of biotechnology in zootechnics is not only a realty, especially in the reproductive area, but will enhance in the next decade, involving more directly the alimental production in the manufacturing level, in the quali-quantitative yield, in the offer's augmentation, in the safety's and productive transparency's increase.

MODERNE BIOTEHNOLOGIJE U STOČARSTVU I REPRODUKCIJI

G. Enne

Rezime

Primena biotehnologija u zootehnici jeste i u budućnosti će sve više predstavljati realnost u strategiji ekonomskog porasta jedne zemlje, jer daju odgovor in a komercijalne i društveno-ekonomkse zahteve (*Madan, 2005*). Biotehnologija u proizvodnji hrane postoji od početka ljudske istorije. Na neki način, evolucija različitih rasa od početka odomaćivanja životinja bi se mogla smatrati biotehnološkim procesom koji traje već 11.000 godina, na bazi potreba u ishrani, društvenih i zahteva životne sredine, sa relativno malim razumevanjem molekularnih procesa (*Wheeler et al., 1993*).

Tokom ovog perioda, sa povećanjem razumevanja i saznanja različitih procesa uključenih u određivanje faktora koji utiču na željene osobine, osvojili smo kontrolu nad tim osobinama. U poslednjem veku, naučnici su lokalizovali u hromozomu nasledne faktore i time odredili bazz

molekularne genetike koja je pojasnila transkripciju i translaciju DNK u proteinu i regulaciju ovih procesa. Istovremeno, razvoj statističkih istraživanja kvantitativnih osobina populacije je korišćen u proizvodnji hrane kako bi se povećalo pojavljivanje i održavanje željenih osobina. Za samo 20 godina brzog razvoja tehnologije rekombinantne DNK, preko embrio tehnologije i genetske manipulacije dobili smo mogućnost kontrole na sve direktniji način onih osobina koje su nam od interesa.

Ključne reči: biotehnologije, stočarstvo, reprodukcija

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