

Animal biotechnology: applications and economic implications in developing countries

M.L. Madan

Livestock Production Systems, 842-sector 6, Urban Estate Karnal, Haryana, 132001, India

Summary

In most developing countries, biotechnological applications relating to livestock need to be suitable for animal owners who are resource-poor small-scale operators who own little or no land and few animals. Livestock is becoming increasingly important to economic growth in developing countries and the application of biotechnology is largely dictated by commercial considerations and socio-economic goals. Using technology to support livestock production is an integral part of viable agriculture in multi-enterprise systems. Livestock are part of a fragile ecosystem and a rich source of animal biodiversity, as local species and breeds possess genes and traits of excellence. Molecular markers are increasingly being used to identify and select the particular genes that lead to these desirable traits and it is now possible to select superior germ plasm and disseminate it using artificial insemination, embryo transfer and other assisted reproductive technologies. These technologies have been used in the genetic improvement of livestock, particularly in cattle and buffaloes, and the economic returns are significant. However, morbidity and mortality among animals produced using assisted reproductive technologies lead to high economic losses, so the principal application of animal biotechnology at present is in the production of cheap and dependable diagnostic kits and vaccines. Several obstacles limit the application of biotechnology at present: there is a lack of infrastructure and insufficient manpower, so funding is needed if resource-poor farmers are to benefit from biotechnology.

Keywords

Biotechnology – Challenge – Constraint – Developing country – Embryo transfer – In vitro fertilisation – Livestock economic – Multi-enterprise system – Reproductive technologies.

Introduction

The developing world is grossly unprepared for the new technological and economic opportunities, challenges and risks that lie on the horizon. Although it is hoped that biotechnology will improve the life of every person in the world and allow more sustainable living, crucial decisions may be dictated by commercial considerations and the socioeconomic goals that society considers to be the most important (37). Globally, livestock production is growing faster than any other sector, and by 2020 livestock is

predicted to become the most important agricultural sector in terms of added value. The use of biotechnology will lead to a distinct shift in the economic returns from livestock. Livestock production currently accounts for about 43% of the gross value of agricultural production (33). In developed countries livestock accounts for more than half of agricultural production, while in developing countries the share is about one-third. This latter share, however, is rising quickly because of rapid increases in livestock production resulting from population growth, urbanisation, changes in lifestyles and dietary habits and increasing disposable incomes.

The livestock economy in developing countries

Livestock is becoming increasingly important in the growth of agriculture in developing economies. The contributions made by livestock to both agriculture and gross domestic product (GDP) have risen (22), at a time when the contribution of agriculture to GDP has fallen (5). The demand for livestock products is a function of income, and sustained growth in per capita income, rising urban populations and changes in diet and lifestyle are fuelling growth in livestock production.

Livestock production contributes to socioeconomic development in many ways, by augmenting income and employment and reducing the incidence of rural poverty (62). Though the role of livestock in ensuring nutritional security is recognised in mixed crop-livestock systems, the importance of livestock goes beyond direct food production. Livestock supply draught power and organic manure to the crop sector, and hides, skins, bones, blood and fibre are used in many industries. Thus, livestock are an important source of income and employment, helping to alleviate poverty and smooth the income distribution among small landholders and the landless, who constitute the bulk of the rural population and the majority of livestock owners. In addition, livestock can easily be converted into cash and thus act as a cushion against crop failure, particularly in less favoured environments. By enabling crop residues and by-products to be used as fodder, livestock production contributes positively to the environment.

Animal owners in the developing world are predominantly resource-poor small-scale operators with little or no land and few animals, who must operate within the constraints of the local climate and who have limited purchasing power and little access to resources or opportunity to determine resource allocation for animal production (35). The situation of the poorest livestock owners is fast deteriorating owing to the fragmentation of limited holdings, exhaustion of land resources and increasing human and animal population pressure (13). Low livestock productivity in many developing countries is considered to reflect, among other things, the inadequate supply of animal husbandry and veterinary services. Veterinary services have traditionally been provided by the State, but financial constraints have limited the availability and effectiveness of public services.

The implications of technology

A major benefit of agricultural research and technology is that the purchasing power of the poor increases, because

both average incomes and access to staple food products are improved. Studies by economists have provided empirical support for the proposition that growth in the livestock sector affects the whole economy (5). Rapid growth of livestock production has stimulated demand for and increased the value of land, labour and non-agricultural goods and services, thereby leading to overall economic growth (14, 19, 28). The poor spend a relatively high proportion of any additional income on food, so increases in livestock production achieved through the use of biotechnology can have major nutritional implications, particularly if the technology is aimed at the poorest producers (1). However, studies have revealed that the commercialisation of agriculture has reduced the nutritional security of the poor (30, 44).

Once production of milk, meat or eggs has been enhanced through the use of technology, it is hoped that it will also make a significant difference in other areas such as nutrition, prevention of diseases, healthcare and other management practices. It is in these areas that biotechnology shows promise and is currently being used. Green Revolution technologies (i.e. those technologies designed to improve the efficiency of agricultural processes and increase crop productivity by relying on the extensive use of chemical fertilizers/pesticides and heavy machinery) are intended to be used in package form (e.g. new plant varieties supplied with recommendations on fertilizer, pesticide and herbicide rates and water control measures); however, among livestock producers many components of these technologies have been taken up in a piecemeal, often stepwise, manner (7). The sequence of adoption is determined by availability and by the potential cost savings. The sequential adoptions of crop management technologies for rice (29) and wheat (64) have been assessed in detail, but few similar studies have focussed on livestock production in developing countries.

Evidence from the People's Republic of China (53), Mexico (65), South Africa (4) and India (6) suggests that small farmers have had no more difficulty than larger farmers in adopting the new technologies. The question, therefore, is not whether biotechnology can benefit small-scale resource-poor farmers, but rather how biotechnology can address the agricultural problems faced by farmers in developing countries. Biotechnology is a promising new tool in the development of applied agricultural technologies. The challenge is to focus this potential on the problems experienced by developing countries.

The introduction of multi-enterprise systems or, more broadly, agricultural diversification is seen as the way forward for agriculture in the developing world; such systems could lift small-scale and marginal farmers out of poverty (17). For example, rotating rice and wheat cropping with dairy farming yields higher profits (56). Introducing multi-enterprise systems involving livestock

enhances the purchasing power of farmers and helps them to obtain nutritional security. It also generates rural (both farming and non-farming) employment, thus preventing excessive migration to urban areas, which is a common problem in developing economies. Multi-enterprise systems also support the natural environment and contribute to capital formation, thus leading to higher overall growth in the agricultural economy. The technology, infrastructure and institutions now exist to make the application of biotechnology in the context of a multi-enterprise system involving livestock production economically viable (56).

Global advantage from livestock of developing countries

The multiplicity of genes, species, populations and agro-ecosystems in the developing countries of South and South East Asia, the People's Republic of China, Africa and Latin America is viewed as a valuable resource for the genetic improvement of livestock on a global scale. The livestock in these countries, which are an integral part of a fragile ecosystem, are a rich source of animal biodiversity. Buffaloes, sheep, goats, camels and zebu cattle have adapted to their regional environments over thousands of years and have provided an important source of sustenance for the population of the region (39).

Livestock production in the developing world has a number of advantages over production in more developed countries, for example:

- the unique and valuable production traits of buffaloes, cattle, sheep, goats and camels
- the low-input production system
- the low unit cost of production
- the lean meat produced from sheep, goats and buffaloes
- the considerable biodiversity
- animal breeds that are resistant to stress and to particular diseases
- the ability of the animals to survive on high-roughage feeds
- the potential for biopharmaceutical developments to lead to significant benefits
- the potential for expanding the microbial food, feed and leather industries
- the integrated production system tailored to the local ecology
- the potential for integrating knowledge and industry.

Several genes and desirable traits have been identified in the livestock of developing countries in Asia and Africa

(24), and some of the livestock species and breeds from these countries have become major contributors to the economy of South America. Examples of breeds from the developing world that are particularly important on a global level are:

- buffaloes that produce milk with a high fat content or with the protein quality required to produce mozzarella cheese
- goats from cold dry regions that produce pashmina and toos (the finest wool in the world)
- Black Bengal goats that carry a gene for high prolificacy
- Garole sheep that carry genes for twinning
- Andaman goats that are highly tolerant of salt
- the yak and mithun that are adapted to high altitude
- the camels, sheep and goats that are adapted to a tropical arid environment and can tolerate feed with a high lignin content
- the many species that are resistant to stress or to particular diseases (39).

Economic impact of technologies

The genetic resources possessed by animals in developing countries often affect economic development (57). Traxler (63) has discussed the economic impacts of biotechnological innovations, but the research and policy options (8, 54, 55) need separate consideration. Animal biotechnology is the result of a multistage process, involving research, development, testing and registration, production and marketing. The goal is to develop a technology, process or product that has clear commercial potential and can be commercialised after due testing and regulatory approval. Developing countries find it difficult to develop biotechnology because the facilities or resources needed to complete all of the stages in the process are often lacking (30). However, several technologies from developed countries have been successfully adopted by developing countries (57).

The impact of technology can be analysed by estimating the growth of total factor productivity (TFP) in livestock production. Not many TFP studies on livestock have been reported. However, separate TFP estimates for the aggregate crop and livestock sectors have been made (49). TFP analysis (36) has shown a shift towards larger, more commercial and more intensive production systems and has further revealed that, as specialisation has developed over the past decade, the importance of backyard livestock production has declined and the importance of specialised household and commercial enterprise has increased. Studies from India (5) have shown that technological input is responsible for about 45% of total output growth and that the TFP growth may be as much as 1.8%.

Technologies that have a specific impact in developing countries

There are a large number of technologies that have been developed for or adapted to the livestock of both developed and developing countries. However, the major technologies that are used effectively in livestock production in the developing world include conserving animal genetic resources, augmenting reproduction, embryo transfer (ET) and related technologies, diagnosing disease and controlling and improving nutrient availability.

Transgenics

Although gene-based technologies have the potential to improve the efficiency of livestock production, thereby ensuring better returns for the farmers, the economic impact of transgenics in the livestock sector will be much less than in the crop sector. However, the global adoption of genetically modified (GM) crops, which were grown on 67.7 million hectares in 2003 compared with 2.8 million hectares in 1996 (32), has had a substantial impact on livestock feed. It is estimated that the United States of America (USA), Argentina, Canada, Brazil and the People's Republic of China have 63%, 21%, 6%, 4% and 4%, respectively, of the global transgenic acreage and that the most frequently grown crops are GM soybean (61%), maize (23%), cotton (11%) and canola (5%) (23). Although few developing countries have released GM crop varieties, a preliminary analysis (16) reveals that more than 20 developing countries are conducting research into the applications of GM crops.

Although transgenic animals (especially mice) are used routinely in research (particularly in the medical field), no GM animals have yet been released on farms. A wide range of traits of potential interest to livestock producers have, however, been the subject of research; for example, the gene responsible for the production of growth hormone (which could be manipulated to increase growth rates), the phytase gene (which could reduce phosphorous emissions from pigs) and keratin genes (which could improve the wool of sheep). The genetic modification of livestock has proceeded much more slowly than the genetic modification of crops for a variety of reasons, including the high costs, the inefficiency of the gene transfer techniques and the low reproductive rates of animals. Recombinant deoxyribonucleic acid (DNA) approaches have been used to promote the expression of desirable genes, to hinder the expression of undesirable genes, to alter specific genes and to inactivate genes so as to block specific pathways. It is estimated that at least 30 enzymes produced by GM bacteria, yeasts and moulds are currently commercially available worldwide; many of these enzymes are used in the food industry.

Genetic engineering has been used to introduce foreign genes into the animal genome or, alternatively, to knock out selected genes. Genes controlling growth were introduced into pigs to increase growth and improve carcass quality. Currently, research is underway to engineer resistance to diseases that affect the animals or that pose an indirect risk to human health, such as Marek's disease and salmonellosis in poultry, scrapie in sheep and mastitis in cattle. Other studies have tried to increase the casein content of milk or to engineer animals that produce pharmaceutical or industrial chemicals in their milk or semen. No agricultural applications have yet proved commercially successful. Nuclear transfer (NT) technology now provides an alternative route for cell-based transgenesis in domestic species, offering new opportunities for genetic modification. Livestock that produce human therapeutic proteins in their milk, that have organs suitable for xenotransplantation and that are resistant to diseases such as spongiform encephalopathies have been produced by NT from engineered cultured somatic cells (15).

Characterising genetic variability

There is considerable genetic diversity in the livestock of developing countries, much of which controls traits that influence adaptability to harsh environments, productivity and susceptibility to disease and parasitism. However, little if any data on these genetic resources are available. Economic analysis can play an important role in ensuring that conservation efforts are appropriately focused (18). The primary challenge facing conservationists is to identify sound reasons why society should preserve animals that livestock keepers have abandoned (45). Jabbar and Diedhiou (31) show that the breeding practices and breed preferences of livestock keepers can successfully be determined by using research techniques such as the revealed preference hedonic approach. On the one hand, conservation cannot be achieved through a conventional breeding programme because the animals carrying the most advantageous traits cannot be easily identified; on the other hand, conservation cannot be achieved through biotechnology because the necessary technologies are either unavailable or uneconomic.

In livestock populations with a high degree of genetic variation, molecular markers are being increasingly used to study the distribution and patterns of genetic diversity. Global surveys indicate that 40% of domestic livestock breeds are at risk of extinction. Most of these breeds are found only in developing countries, and often little is known about them or their potential. Rapid progress is being made in the preparation of dense microsatellite linkage maps to assist in the search for genetic traits of economic importance. These linkage maps can be used to

develop strategies for marker assisted selection and marker assisted introgression that will meet the goals of breeding programmes in developing countries. Molecular markers have been widely used in the identification of genotypes and the 'genetic fingerprinting' of organisms. Genotype verification is used intensively to determine the parentage of domestic animals and to trace livestock products in the food chain back to the farm and animal of origin.

Reproductive technologies

The main objectives of using reproductive biotechnologies in livestock are to increase production, reproductive efficiency and rates of genetic improvement. Over the years, many options have become available for managing the reproduction of the major large and small ruminants. Artificial insemination (AI) and preservation of semen are the main technologies that are used extensively. Assessing the fertilisation capacity of sperms, sexing sperms, synchronisation and fixed-time insemination, superovulation, embryo transfer (ET) and *in vitro* embryo production (IVEP) are additional techniques that can improve reproductive efficiency and pregnancy rates. Reproductive technologies can also be used to control reproductive diseases if procedures and protocols are accurately followed (38).

Artificial insemination

The conception rate in field AI programmes in developing countries is very low, and therefore the desired effect in terms of animal improvement has not been achieved. Most semen banks still evaluate semen on the basis of sperm motility, even though significant advances have been made in techniques for semen evaluation. Although detailed guidelines are available regarding the processing, storage and thawing of cattle semen (67) and buffalo semen (58), the processing and handling procedures in laboratories processing semen are often inadequate. Only when farmers have access to considerably better technical and organisational facilities will AI become more effective. At present, the efficiency of the technology is limited by organisational and logistical constraints and by the failure to provide appropriate training for farmers. Several modifications of the technique have been suggested to increase the conception rate. Synchronisation with different compounds, and the use of gonadotropin-releasing hormone (GnRH) followed seven days later by prostaglandin F_{2α} (PGF_{2α}) can synchronise oestrus and improves the conception rate (59). In this protocol giving injections of GnRH on day 0, PGF_{2α} on day 7 and GnRH on day 9 is called the 'Ovsynch' programme and synchronises ovulation, permitting timed insemination. The ability to control ovarian follicular and corpus luteum development has allowed insemination in cattle to be timed without having to detect oestrus, and this has increased the net revenue per cow.

Embryo transfer

One of the major reproductive technologies that can facilitate genetic improvement in cattle is ET. Unfortunately, commercial ET programmes are limited by the high variability in the ovarian follicular response to gonadotropin stimulation. Multiple ovulation and embryo transfer (MOET) takes AI one step further, in terms of both the possible genetic gains and the level of technical expertise and organisation required. In 2001, 450,000 embryos were transferred globally, mainly in dairy cattle, with 62% being transferred in North America and Europe, 16% in South America and 11% in Asia. The main potential advantage of MOET for developing countries is that the elite females of local breeds can be identified, and bulls can be produced from them for use in a field programme of breed improvement.

Zebu cattle and buffaloes in developing countries exhibit less consistent follicular dynamics after superovulation than *Bos taurus* in the developed world (2). However, over the last 10 to 15 years, the number of transferable embryos produced by zebu donors has increased from 2.4 to 5.8 embryos per flush in the late 1980s to 5.6 to 9.9 embryos per flush in 2000 (2). The use of ET (46, 61) has been less successful than envisaged for several reasons. The low reproductive efficiency (60), poor superovulatory responses (43), very low primordial follicle population and high incidence of atresia (39) all contribute to low embryo production. In buffaloes, embryo recovery was initially less than one, but has subsequently improved to 2.6 with 1.4 transferable embryos per flush (40). After transferring buffalo embryos to recipients, the conception rate is only 16% (61). The poor success rates have limited the use of ET in buffaloes, which are the main dairy animals in developing countries in Asia, South-East Asia and the Mediterranean region.

In vitro production of embryos

Since the birth of the first buffalo calf from an *in vitro* fertilised oocyte (40), a number of publications have described the effects of different protocols and media on oocyte and embryo development. Two extensive reviews have been published recently (26, 48). However, the practical use of IVEP is limited by high production costs and the low overall efficiency under field conditions. High rates of maturation (70% to 90%), fertilisation (60% to 70%) and cleavage (40% to 50%), and moderate to low rates of blastocyst formation (15% to 30%) and calf production (10.5%) have been reported in the literature (48). The efficiency of blastocyst production in buffaloes is much poorer than the 30% to 60% reported for cattle (20). Although viable buffalo blastocysts have been produced from ovaries obtained from abattoirs (41, 42), the yield of transferable embryos remains low (15% to 39%) (9, 10, 11, 47, 48). Embryos produced *in vitro* have led successfully to pregnancy and calf birth in buffalo

(9, 25, 41), but the success rate is low. Therefore IVEP must be improved before it can be widely used in cattle and buffaloes in developing countries.

Improving health through developing vaccines

Most biotechnologies related to health focus on the needs of the developed world, meaning that 90% of health research is devoted to the health problems of 10% of the world's population (12). Two main approaches are being used to develop vaccines using recombinant DNA technology. The first involves deleting genes that determine the virulence of the pathogen, thus producing attenuated organisms (non-pathogens) that can be used as live vaccines. Currently, this strategy is more effective against viral and bacterial diseases than against parasites. Attenuated live vaccines have been developed against the herpes viruses that cause pseudorabies in pigs and infectious bovine rhinotracheitis in cattle. A number of candidate *Salmonella* vaccines have also been produced. The second approach is to identify protein subunits of pathogens that can stimulate immunity. The International Livestock Research Institute (ILRI) used this approach to develop a vaccine against *Theileria parva*, the parasite that causes East Coast fever in African cattle.

A novel strategy for developing vaccines against blood-sucking parasites involves using components of the gut wall of the parasite that are not usually exposed to the immune system of the host. When the parasite feeds, it ingests antibodies induced by the vaccine, which destroy the gut wall and, consequently, kill the parasite. This strategy has been used successfully to develop a vaccine against the one-host tick *Boophilus microplus*.

Vaccination is one of the most effective and sustainable methods of controlling disease (33, 34). Vaccines against parasitic diseases in Africa and viral diseases in Asia have been shown to control disease effectively and increase livestock productivity. A recent approach has been to use vaccines based on DNA (66). The use of DNA in vaccines is based on the discovery that injecting genes in the form of plasmid DNA can stimulate an immune response to the respective gene products. This immune response is a result of the genes being taken up and expressed by cells in the animal after injection. The live-vector and DNA vaccination systems could be manipulated further to enhance the immunity conferred by the gene products. Experimental studies have demonstrated that these vaccines can potentially induce appropriate and enduring immune responses. This technology is, in principle, one of the simplest and yet most versatile methods of inducing both humeral and cellular immune responses, as well as protecting against a variety of infectious agents. However, although immune responses have been induced in a number of larger species, most of the information on the

efficacy of DNA immunisation comes from studies of mice. An exhaustive review of the information available on the use of DNA vaccines in farm animals, including cattle, pigs and poultry, has identified the areas that need specific attention before this technology can be used routinely (37). These areas include the delivery, safety and compatibility of plasmids in multivalent vaccines and the potential for using immune stimulants as part of a DNA vaccine. Korean scientists have developed a combined vaccine against pleuropneumonia, pneumonic pasteurellosis and enzootic pneumonia in swine (50). Molecular biology has been used to produce an improved vaccine against swine fever. In the Philippines, a vaccine has been developed that protects cattle and water buffalo against haemorrhagic septicaemia, which is the leading cause of death in these animals. The new vaccine provides improved protection at a very low cost.

Diagnostics and epidemiology

Advanced diagnostic tests that use biotechnology enable the agents causing disease to be identified and the impact of disease control programmes to be monitored more precisely than was previously possible. Molecular epidemiology characterises pathogens (viruses, bacteria, parasites and fungi) by nucleotide sequencing, enabling their origins to be traced. This is particularly important for epidemic diseases, in which pinpointing the source of the infection can significantly improve disease control. For example, the molecular analysis of rinderpest viruses has been vital in determining the lineages circulating in the world and instrumental in aiding the Global Rinderpest Eradication Programme. Enzyme-linked immunosorbent assays have become the standard means of diagnosing and monitoring many animal and fish diseases worldwide, and the PCR technique is especially useful in diagnosing livestock disease.

Many diagnostic techniques currently used in developing countries are cumbersome and unsuitable for low-resource settings. Molecular diagnostic technologies that are either already in use or being tested in low-income regions include polymerase chain reaction (PCR), monoclonal antibodies and recombinant antigens. These technologies can be modified to facilitate their application in the developing world (12). Simple hand-held devices that rely on the binding specificity of monoclonal antibodies or recombinant antigens to diagnose infection may be easily adapted for use in settings without running water, refrigeration or electricity.

Molecular characterisation of the virus serotypes causing foot and mouth disease has helped in the vaccination and control programmes in Asia. In Japan and Taiwan, DNA testing is being used to diagnose hereditary weaknesses of livestock (50). One test looks for the presence of the gene

responsible for porcine stress syndrome in pigs. Pigs with this gene tend to produce pale poor-quality meat because of their reaction to the stress of transport and slaughter. Pigs with this gene can now be excluded from breeding programmes, so the gene will become less common. In addition, DNA testing is being used in Japan to check for the gene that causes leucocyte adhesion deficiency in Holstein cattle. Cattle with this condition suffer from gum disease, tooth loss and stunted growth. They usually die before they are one year old. By using DNA testing, carriers can be identified and eliminated from breeding herds. Bulls used for breeding can also be tested to make sure that they are not carriers. Another DNA test identifies a gene that leads to anaemia and retarded growth in Japanese Black cattle.

Nutrition and feed utilisation

The shortage of feed in most developing countries and the increasing cost of feed ingredients mean that there is a need to improve feed utilisation. Aids to animal nutrition, such as enzymes, probiotics, single-cell proteins and antibiotics in feed, are already widely used in intensive production systems worldwide to improve the nutrient availability of feeds and the productivity of livestock. Gene-based technologies are being increasingly used to improve animal nutrition, either through modifying the feeds to make them more digestible or through modifying the digestive and metabolic systems of the animals to enable them to make better use of the available feeds (3, 27). Feeds derived from GM plants (a quarter of which are now grown in developing countries), such as grain, silage and hay, have contributed to increases in growth rates and milk yield. Genetically modified crops with improved amino acid profiles can be used to decrease nitrogen excretion in pigs and poultry. Increasing the levels of amino acids in grain means that the essential amino acid requirements of pigs and poultry can be met by diets that are lower in protein.

Metabolic modifiers have also been used to increase production efficiency (weight gain or milk yield per feed unit), improve carcass composition (meat-fat ratio), increase milk yield and decrease animal fat. The use of recombinant bovine somatotropin (rBST) in dairy cows increases both milk yield and production efficiency and decreases animal fat. In the USA, the use of rBST typically increases milk yield by 10% to 15%. Although trials conducted in developing countries have reported a similar percentage increase, this increase is not significant because of the low milk yields and the high cost-benefit ratio. However, rBST is being used commercially in 19 countries where the economic returns make its use worthwhile. A porcine somatotropin has been developed that increases muscle growth and reduces body-fat deposition, resulting in pigs that are leaner and of greater market value.

Constraints on applying the technology

The application of new molecular biotechnologies and new breeding strategies to the livestock breeds used in smallholder production systems in developing countries is constrained by a number of factors. In the developing world, poverty, malnutrition, disease, poor hygiene and unemployment are widespread, and biotechnologies must be able to be applied in this context. Over the last few decades, the green revolution has brought comparative prosperity to farmers with land, but the majority of farmers, who are landless or marginal farmers and subsist only on livestock, have been neglected and remain poor.

The major constraints on applying biotechnologies have been enumerated by Madan (39) and include:

- a) the absence of an accurate and complete database on livestock and animal owners so that programmes can be implemented
- b) the biodiversity present within species and breeds in agro-ecological systems
- c) the fact that models of biotechnological intervention differ distinctly between developed and developing economies
- d) the fact that many animal species and breeds are unique to the developing world; each has its own distinct developmental, production, disease resistance and nutrient utilisation characteristics
- e) the lack of trained scientists, technicians and fieldworkers to develop and apply the technologies, both in the government and in the private sectors
- f) the absence of an interface between industry, universities and institutions, which is necessary to translate technologies into products
- g) the inability to access technologies from the developed world at an affordable price in order to make a rightful, positive and sustainable contribution to livestock production and the economic welfare of farmers
- h) the high cost of technological inputs such as materials, biologicals and equipment
- i) the failure to address issues of biosafety and to conduct risk analyses of new biologicals, gene products, transgenics and modified food items, and, above all
- j) the negligible investment in animal biotechnology.

The critical issues affecting livestock productivity have recently been re-examined. Research that aims to enhance productivity and sustainability should focus on improving

livestock feeds and nutrition, improving animal health, managing natural resources relating to the livestock sector, assessing the impact of technological interventions, and strengthening the capacity of the national agricultural research systems of developing countries (24). Furthermore, the potential production capacity and contribution of livestock to the economy are still not being achieved in developing countries because the transfer, adaptation and adoption of technology is hampered by the lack of a clear policy for livestock development that is conducive to the introduction of new proven technology and by the lack of information flow from and to decision makers.

In developing countries, there is a wealth gap between urban and rural areas, which persists and may even be widening; the rural-urban divide also tends to be reflected in education and health indicators (23). In addition, women in rural (and urban) areas who are predominantly involved in animal husbandry have higher illiteracy rates than men (21). A survey of 21 African countries recently highlighted the substantial disparities in primary schooling between urban and rural areas, in favour of urban dwellers. Special attention must be given to the knowledge and information needed to enable rural people to apply biotechnology. There is a need to identify alternative delivery systems (beyond the State) for animal healthcare and to propose new roles for the state and the private sector in service delivery.

Building capacity

Owing to the constraints outlined above, the economic benefits of animal biotechnology cannot be realised without a conscious, sustained, holistic, multi-stakeholder, participatory approach. There is a great need to ensure that capacity is not just created but also is retained and enhanced. Capacity-building activities must be carried out at all levels: the awareness of policy and decision makers must be raised, the necessary legal and regulatory frameworks must be initiated, the technical and regulatory capacities must be enhanced and institutions may need to be overhauled. More importantly, it is necessary to assess and deploy competent operators and institutional capacity continuously so that, as biotechnology advances, the procedures required for its safe use can be constantly evaluated, upgraded and applied. This is a daunting task, but it can be achieved through firm commitment and partnerships.

Funding to implement technology

Developing and commercialising improved technologies in most developing countries has been the responsibility of the public sector, and technology has been disseminated

freely (51). This situation will have to continue if superior genetics, diagnostics and vaccines are to be delivered. However, research and almost all commercial development of biotechnology in the developed world are being driven largely by the private sector (52).

The global trends in funding for research and development and production do not address the concerns, needs and opportunities of the developing world. Developing countries are finding it increasingly expensive to access and use new technologies. There is limited private- and public-sector investment in animal health and production, particularly in relation to modern biotechnologies that are 'resource hungry'. Although several discoveries have been made in laboratories in the developing world, in most cases these have not been converted into useful technologies or products. The key potential users – resource-poor often illiterate farmers with a limited knowledge base – do not feel that applying these technologies is worth the effort, cost and risk involved. This is mainly because there is no agency or industry that can scale up and package the technology. Also, in the developed world, there is an economic incentive to market biotechnological services and products; this is lacking in the developing world because of the limited purchasing power of resource-poor stakeholders. Research in biotechnology in recent years has also been motivated by economic considerations, and little research is conducted in the developing world because of the probable lack of returns on the investment. For understandable reasons, current funding policies in developing countries focus on areas that will yield practical benefit in the short term. In determining future policy, policy-makers and funding bodies must not lose sight of the substantial benefits that can be gained in the longer term by investing in strategic research into vaccine development.

Adequate multi-institutional (national and international) support through an international donor consortium is needed to develop cost-effective, cheap and easily adaptable biotechnological products. The amount spent by international agencies on animal biotechnology in developing countries is currently very low and constitutes only a small percentage of the total spending on agriculture. The World Bank, the Food and Agriculture Organization, the Consultative Group on International Agricultural Research, the United Nations Development Programme, the United States Agency for International Development, the Swedish International Development Cooperation Agency, the International Development Research Centre, the Asian Development Bank and other donor and funding agencies have to designate a higher percentage of funds to the livestock sector (39). It has been convincingly shown that investing in livestock has a dramatic and far-reaching impact on the human development index. This is a strong argument in

favour of investing heavily in animal production and health biotechnologies in order to bring economic prosperity, nutritional security, rural development and health improvements to poor populations in the developing world.

Conclusions

Although animal production is being changed significantly by advances made in thousands of biotechnology laboratories around the world, benefits are reaching the developing world in only a few areas of conservation, animal improvement, healthcare (including diagnosis and

control of disease) and the augmentation of feed resources. Adopting biotechnology has resulted in distinct benefits in terms of animal improvement and economic returns to the farmers. Over the past decade, the ILRI has focused on biotechnological applications, especially in Africa, and several developing countries now have multi-institutional programmes to develop and apply biotechnology. The developing world will have to respond to the many gene-based technologies now being developed with a sense of commitment, trained manpower, infrastructure and funding. ■

Biotechnologie animale : applications et implications économiques dans les pays en développement

M.L. Madan

Résumé

Dans la plupart des pays en développement, les applications biotechnologiques concernant l'élevage doivent être accessibles aux éleveurs dont l'exploitation est de petite taille et dont les ressources sont limitées, qui possèdent quelques animaux et peu ou pas de terres. Or le bétail devient de plus en plus important pour la croissance économique des pays en développement et l'application de la biotechnologie est en grande partie dictée par des considérations commerciales et des objectifs socio-économiques. Le recours à la technologie pour soutenir la production animale fait partie intégrante d'une agriculture viable dans le cadre de systèmes de production diversifiés multi-entreprises. Les animaux d'élevage font partie d'un écosystème fragile et constituent une source importante de biodiversité animale puisque les espèces et les races locales possèdent des gènes et des caractères d'excellence. Les marqueurs moléculaires sont de plus en plus utilisés pour identifier et sélectionner les gènes qui déterminent ces caractères recherchés et il est désormais possible de sélectionner un germoplasme supérieur et de le disséminer par insémination artificielle, par transfert d'embryon et autres techniques de reproduction assistée. Ces technologies ont été employées pour l'amélioration génétique du bétail, en particulier des bovins et des buffles, et leurs bénéfices économiques sont importants. Toutefois, comme la morbidité et la mortalité observées parmi les animaux produits par des techniques de reproduction assistée se traduisent par de lourdes pertes économiques, la principale application de la biotechnologie animale est actuellement axée sur la production de kits de diagnostic et de vaccins peu coûteux et fiables. Plusieurs obstacles limitent aujourd'hui l'application de la biotechnologie : le manque d'infrastructures et les ressources humaines insuffisantes. Des financements sont donc nécessaires pour que les éleveurs disposant de ressources limitées puissent bénéficier de la biotechnologie.

Mots-clés

Biotechnologie – Contrainte – Défi – Économie de l'élevage – Fécondation in vitro – Pays en développement – Système multi-entreprises – Techniques de la reproduction. ■

Aplicaciones de la biotecnología al mundo animal y repercusiones económicas en los países en desarrollo

M.L. Madan

Resumen

En la mayoría de los países en desarrollo, las aplicaciones de la biotecnología relacionadas con el ganado deben ser apropiadas para pequeños propietarios que trabajan con pocos recursos y a pequeña escala, tienen pocos animales y poseen, en el mejor de los casos, exiguas parcelas de tierra. El ganado bovino es cada vez más importante para el crecimiento económico de los países en desarrollo, y la aplicación de la biotecnología a esos animales está supeditada en gran medida a consideraciones de rentabilidad y objetivos socioeconómicos. El uso de la biotecnología en apoyo de la producción ganadera forma parte integral de una agricultura viable en sistemas multiempresariales. Los animales forman parte del frágil ecosistema en el que viven y son un rico reservorio de diversidad biológica, no en vano las especies y razas locales atesoran genes y rasgos de excelencia. Los marcadores moleculares se utilizan cada vez con más frecuencia para localizar y seleccionar los genes concretos portadores de esos rasgos deseables. Hoy en día ya es posible seleccionar germoplasma superior y diseminarlo con procedimientos de inseminación artificial, transferencia de embriones y demás técnicas de reproducción asistida. Estas técnicas, que se han utilizado para la mejora genética del ganado, en particular bovinos y búfalos, ofrecen un importante rendimiento económico. Sin embargo, la morbilidad y mortalidad de animales obtenidos con técnicas de reproducción asistida provocan elevadas pérdidas financieras, por lo que hoy en día la principal aplicación de la biotecnología al mundo animal es la elaboración de vacunas y kits de diagnóstico baratos y fiables. Entre los varios obstáculos que de momento frenan la aplicación de la biotecnología destacan la falta de infraestructura y la escasez de recursos humanos. Para que los granjeros con pocos recursos puedan beneficiarse de la biotecnología se necesitan por consiguiente medios financieros.

Palabras clave

Biotecnología – Desafío – Economía ganadera – Fertilización in vitro – Limitación – País en desarrollo – Sistema multiempresarial – Técnica de reproducción – Transferencia de embriones.



References

1. Alston J.M., Norton G.W. & Pardey P.G. (1995). – Science under scarcity: principles and practice for agricultural research evaluation and priority setting. Cornell University Press, Ithaca, 585 pp.
2. Barros C.M. & Nogueira M.F.G. (2001). – Embryo transfer in *Bos indicus* cattle. *Theriogenology*, **56**, 1483-1496.
3. Bedford M.R. (2000). – Exogenous enzymes in monogastric nutrition: their current value and future benefits. *Anim. Feed Sci. Technol.*, **86**, 1-13.
4. Bennett R., Morse S. & Ismael Y. (2003). – The benefits of Bt cotton to small-scale producers in developing countries: the case of South Africa. In 7th ICABR International Conference on Public Goods and Public Policy for Agricultural Biotechnology, 29 June-3 July, Ravello. International Consortium on Agricultural Biotechnology Research, Ravello. Website: www.economia.uniroma2.it/conferenze/icabr2003/papers/index.htm (accessed on 1 June 2005).
5. Birthal P.S., Kumar A., Ravi Shankar A. & Pandey U.K. (1999). – Sources of growth in the livestock sector. Policy paper No. 9. National Centre for Agricultural Economics and Policy Research, New Delhi, 58 pp.

6. Birthal P.S., Joshi P.K. & Kumar A. (2002). – Assessment of research priorities for livestock sector in India. Policy paper No. 15. National Centre for Agricultural Economics and Policy Research, New Delhi, 64 pp.
7. Byerlee D. & Hesse de Polanco E. (1986). – Farmers' stepwise adoption of technological packages: evidence from the Mexican Altiplano. *Am. J. agric. Econ.*, **68**, 519-527.
8. Byerlee D. & Fischer K. (2002). – Accessing modern science: policy and institutional options for agricultural biotechnology in developing countries. *World Dev.*, **30**, 931-948.
9. Chauhan M.S., Singla S.K., Manik R.S. & Madan M.L. (1997). – Increased capacitation of buffalo sperm by heparin as confirmed by electron microscopy and *in vitro* fertilization. *Indian J. experim. Biol.*, **35**, 1038-1043.
10. Chauhan M.S., Singla S.K., Palta P., Manik R.S. & Madan M.L. (1998). – *In vitro* maturation and fertilization, and subsequent development of buffalo (*Bubalus bubalis*) embryos: effects of oocyte quality and type of serum. *Reprod. Fertil. Dev.*, **10**, 173-177.
11. Chauhan M.S., Singla S.K., Palta P., Manik R.S. & Madan M.L. (1999). – Effect of epidermal growth factor on the cumulus expansion, meiotic maturation and development of buffalo oocytes *in vitro*. *Vet. Rec.*, **144**, 266-267.
12. Daar A.S., Thorsteinsdottir H., Martin D.K., Smith A.C., Nast S. & Singer P.A. (2002). – Top ten biotechnologies for improving health in developing countries. *Nature Genet.*, **32**, 229-232.
13. Dastagiri M.B. (2004). – Demand and policy projections for livestock production in India. Policy paper No. 21. National Centre for Agricultural Economics and Policy Research, New Delhi.
14. Delgado C.L., Hopkins J. & Kelly V.A. (1998). – Agricultural growth linkages in sub-Saharan Africa. Research Report No. 107, International Food Policy Research Institute, Washington, DC, 154 pp.
15. Denning C. & Priddle H. (2003). – New frontiers in gene targeting and cloning: success, application and challenges in domestic animals and human embryonic stem cells. *Reproduction*, **126**, 1-11.
16. Dhalmini Z., Spillane C., Moss J.P., Ruane J., Urquia N. & Sonnino A. (2005). – Status of research and application of crop biotechnologies in developing countries – a preliminary assessment. Renouf Books, Ogdensburg, New York, 62 pp.
17. Dorjee K., Broca S. & Pingali P. (2003). – Diversification in South Asian agriculture: trends and constraints. ESA Working paper 03-15. Food and Agriculture Organization, Rome, 23 pp.
18. Drucker A.G. (2004). – The economics of farm animal genetic resource conservation and sustainable use: why is it important and what have we learned? Background study paper No. 21. Food and Agriculture Organization, Rome, 11 pp.
19. Fan S., Hazell P. & Thorat S. (1998). – Government spending, growth and poverty: an analysis of interlinkages in rural India. Environment and Production Technology Division discussion paper No. 33, International Food Policy Research Institute, Washington, DC, 92 pp.
20. Farin P.W., Crosier A.E. & Farin C.E. (2001). – Influence of *in vitro* systems on embryo survival and fetal development in cattle. *Theriogenology*, **55**, 151-170.
21. Flavell R. (1999). – Biotechnology and food and nutrition needs: biotechnology for developing-country agriculture: problems and opportunities, Brief 2 of 10. International Food Policy Research Institute, Washington, DC, 2 pp.
22. Food and Agriculture Organization (FAO) (2000). – The appropriateness, significance and application of biotechnology options in the animal agriculture of developing countries. Electronic forum on biotechnology in food and agriculture. Conference 3. FAO, Rome.
23. Food and Agriculture Organization (FAO) (2004). – The State of Food and Agriculture 2003-2004. Agricultural biotechnology: meeting the needs of the poor. FAO, Rome, 209 pp.
24. Food and Agriculture Organization (FAO)/International Atomic Energy Agency (IAEA) (2004). – Final report – international symposium on applications of gene-based technologies for improving animal production and health in developing countries. FAO/IAEA, Vienna, 21 pp.
25. Galli C., Crotti G., Notari C., Turini P., Duchi R. & Lazzari G. (2001). – Embryo production by ovum pick up from live donors. *Theriogenology*, **55**, 1341-1357.
26. Gasparri B. (2002). – *In vitro* embryo production in buffalo species: state of the art. *Theriogenology*, **57**, 237-256.
27. Gordon G.L.R. & Phillips M.W. (1998). – The role of anaerobic gut fungi in ruminants. *Nutr. Res. Rev.*, **11**, 133-168.
28. Hazell P. & Haggblade S. (1993). – Farm-nonfarm growth linkages and the welfare of the poor. In Including the poor (M. Lipton & J. van der Gaag, eds). World Bank, Washington, DC, 190-204.
29. Herdt R.W. (1987). – A retrospective view of technological and other changes in Philippine rice farming 1965-1982. *Econ. Dev. cult. Change*, **35**, 329-349.
30. Hobbelink H. (1987). – New hope or false promise? Biotechnology and Third World agriculture. International Coalition for Development Action, Brussels, 72 pp.
31. Jabbar M.A. & Diedhiou M.L. (2003). – Does breed matter to cattle farmers and buyers? Evidence from West Africa. *Ecol. Econ.*, **45**, 461-472.
32. James C. (2003). – Preview: global status of commercialized transgenic crops 2003. International Service for the Acquisition of Agri-biotech Applications (ISAAA) Briefs No. 30. ISAAA, Ithaca, New York, 8 pp.

33. Jutzi S. (2003). – Applications of gene-based technologies for improving animal production and health in developing countries. FAO/IAEA International Symposium, Vienna, Austria, 6-10 October 2003. Opening address. Food and Agriculture Organization/International Atomic Energy Agency, Vienna. Website: www.iaea.org/programmes/nafa/d3/public/opening-address-director-fao.pdf.
34. Kurstak E. (1999). – Towards new vaccines and modern vaccinology: introductory remarks. *Vaccine*, **17**, 1583-1586.
35. McDermott J.J., Randolph T.F. & Staal S.J. (1999). – The economics of optimal health and productivity in smallholder livestock systems in developing countries. In *The economics of animal disease control*. Rev. sci. tech. Off. int. Epiz., **18** (2), 399-424.
36. Ma H., Rae A.N. & Huang J. (2004). – Livestock productivity in China: data revision and total factor productivity decomposition, China agriculture working paper 1/04, Centre for Applied Economics and Policy Studies. Massey University, Palmerston North, 31 pp.
37. Macer D.R.J. (1996). – Biotechnology, international competition, and its economic ethical and social implications in developing countries. In *Concepts in biotechnology* (D. Balasubramanian, C.F.A. Bryce, K. Dharmalingam, J. Green, K. Jayaraman, ed.). Universities Press Pvt. Ltd. Orient Longman Inc., Hyderabad, 378-397.
38. Madan M.L. (2002). – Biotechnologies in animal reproduction. Key note address at international conference on animal biotechnology. Tamilnadu Veterinary and Animal Science University, Chennai.
39. Madan M.L. (2003). – Opportunities and constraints for using gene-based technologies in animal agriculture in developing countries and possible role of international donor agencies in promoting R&D in this field. In *FAO/IAEA international symposium on applications of gene-based technologies for improving animal production and health in developing countries*, Vienna, Austria, 6-10 October 2003. Food and Agriculture Organization/International Atomic Energy Agency, Vienna, 103-104.
40. Madan M.L., Singla S.K., Jaikhani S. & Ambrose J.D. (1991). – *In vitro* fertilization and birth of first ever IVF buffalo calf. In *Proc. 3rd World Buffalo Congress*, Varna, 11-17.
41. Madan M.L., Singla S.K., Chauhan M.S. & Manik R.S. (1994). – *In vitro* production and transfer of embryos in buffaloes. *Theriogenology*, **41**, 139-143.
42. Madan M.L., Chauhan M.S., Singla S.K. & Manik R.S. (1994). – Pregnancies established from water buffalo (*Bubalus bubalis*) blastocysts derived from *in vitro* matured, *in vitro* fertilized oocytes and co-cultured with cumulus and oviductal cells. *Theriogenology*, **42**, 591-600.
43. Madan M.L., Das S.K. & Palta P. (1996). – Application of reproductive technology to buffaloes. *Anim. Reprod. Sci.*, **42**, 299-306.
44. Mellor J.W. (1997). – The new economics of growth: 40 years of agricultural development; what is old, what is new. *Asian J. Agric. Econ.*, **2**, 123-129.
45. Mendelsohn R. (2003). – The challenge of conserving indigenous domesticated animals. *Ecol. Econ.*, **45**, 501-510.
46. Misra A.K. (1997). – Application of biotechnologies to buffalo breeding in India. In *3rd Course on biotechnology of reproduction in buffaloes*, Caserta, 141-166.
47. Nandi S., Chauhan M.S. & Palta P. (1998). – Influence of cumulus cells and sperm concentration on cleavage rate and subsequent embryonic development of buffalo (*Bubalus bubalis*) oocytes matured and fertilized *in vitro*. *Theriogenology*, **50**, 1251-1262.
48. Nandi S., Raghu H.M., Ravindranatha B.M. & Chauhan M.S. (2002). – Production of buffalo (*Bubalus bubalis*) embryos *in vitro*: premises and promises. *Reprod. dom. Anim.*, **37**, 65-74.
49. Nin A., Hertel T.W., Rae A.N. & Ehui S. (2002). – Productivity growth, 'catching up' and trade in livestock products. ILRI Socio-economics and policy research working paper No. 37. International Livestock Research Institute, Nairobi, 41 pp.
50. Oishi T., Cheong I.C., Villar E.C., Lee S.N. & Vajrabukka C. (1999). – Applied biotechnology in animal production. Issues in Asian Agriculture 1999-06-01. Food and Fertilizer Technology Center, Taiwan. Website: www.ffc.agnet.org (accessed on 2 June 2005).
51. Pal S. & Byerlee D. (2003). – The funding and organization of agricultural research in India: evolution and emerging policy issues. Policy paper No. 16. National Centre for Agricultural Economics and Policy Research, New Delhi, 29 pp.
52. Pingali P.L. & Traxler G. (2002). – Changing locus of agricultural research: will the poor benefit from biotechnology and privatization trends? *Food Policy*, **27**, 223-238.
53. Pray C.E. & Huang J. (2003). – The impact of BT cotton in China. In *The economic and environmental impacts of agbiotech: a global perspective* (N. Kalaitzandonakes, ed.). Kluwer-Plenum Academic Publishers, New York, 223-242.
54. Pray C.E. & Naseem A. (2003). – The economics of agricultural biotechnology research. ESA working paper No. 03-07. Food and Agriculture Organization, Rome, 37 pp.
55. Pray C.E. & Naseem A. (2003). – Biotechnology R&D: policy options to ensure access and benefits for the poor. ESA working paper No. 03-08. Food and Agriculture Organization, Rome, 37 pp.
56. Rangi P.S. (2004). – Trade and policy issues and development of multi-enterprise agriculture system. Multi-enterprise system for viable agriculture (C.L. Acharya, R.K. Gupta, D.N.L. Rao & A. Subba Rao, eds). In *Proc. 6th Agricultural Science Congress*, Bhopal, 13-15 February 2003. National Academy of Agricultural Sciences, New Delhi, 315-328.
57. Rege J.E.O. & Gibson J.P. (2003). – Animal genetic resources and economic development: issues in relation to economic valuation. *Ecol. Econ.*, **45**, 319-330.

58. Sansone G., Nastri M.J.F. & Fabbrocini A. (2000). – Storage of buffalo (*Bubalus bubalis*) semen. *Anim. Reprod. Sci.*, **62**, 55-76.
59. Schmitt E.J., Diaz T., Drost M. & Thatcher W.W. (1996). – Use of a gonadotropin-releasing hormone agonist or human chorionic gonadotropin for timed insemination in cattle. *J. Anim. Sci.*, **74**, 1084-1091.
60. Singh J., Nanda A.S. & Adams G.P. (2000). – The reproductive pattern and efficiency of female buffaloes. *Anim. Reprod. Sci.*, **60-61**, 593-604.
61. Singla S.K., Manik R.S. & Madan M.L. (1996). – Embryo biotechnology in buffaloes: a review. *Bubalus bubalis*, **1**, 53-63.
62. Taneja V.K. & BIRTHAL P.S. (2004). – Animal husbandry entrepreneurship and policy support. Multi-enterprise system for viable agriculture (C.L. Acharya, R.K. Gupta, D.N.L. Rao & A. Subba Rao, eds). *In Proc. 6th Agricultural Science Congress, Bhopal, 13-15 February 2003*. National Academy of Agricultural Sciences, New Delhi, 85-100.
63. Traxler G. (2004). – The economic impacts of biotechnology-based technological innovations. ESA working paper No. 04-08. Food and Agriculture Organization, Rome, 27 pp.
64. Traxler G. & Byerlee D. (1992). – Economic returns to crop management research in post-green revolution setting. *Am. J. agric. Econ.*, **74**, 573-582.
65. Traxler G., Godoy-Avila S., Falck-Zepeda J. & Espinoza-Arellano J.J. (2003). – Transgenic cotton in Mexico: a case study of the Comarca Lagunera. *In The economic and environmental impacts of agbiotech: a global perspective* (N. Kalaitzandonakes, ed.). Kluwer-Plenum Academic Publishers, New York, 183-202.
66. Van Drunen Littel-van den Hurk S., Gerdtz V., Loehr B.I., Pontarollo R., Rankin R., Uwiera R. & Babiuk L.A. (2000). – Recent advances in the use of DNA vaccines for the treatment of diseases of farmed animals. *Adv. Drug Deliv. Rev.*, **43**, 13-28.
67. Vishwanath R. & Shannon P. (2000). – Storage of bovine semen in liquid and frozen state. *Anim. Reprod. Sci.*, **62**, 23-53.
-

