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Review

Genetic engineering in animal production: Applications and prospects

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ABSTRACT

Genetic engineering is the manipulation of genetic material, that is, DNA and/or RNA with the objective of bringing about any desired change or innovation, either *in vitro* or *in vivo*, as carried out during the study or modification for any purpose, of genes or genetic systems. Genetic engineering therefore includes, for example, those *in vitro* techniques involved in the study of genes and their regulation; various techniques used in gene therapy; and the creation of novel strains of existing microorganisms for medical or industrial use. It also includes a group of techniques used for modification of organisms. The techniques permit individual or group of genes to be isolated from large masses of DNA and produced in virtually unlimited quantities. This is through recombining DNA fragments from one organism and transferring them to another for expression. The hybrid molecule formed when a fragment of DNA from one organism is spliced to another DNA fragment is called recombinant DNA. Genetic engineering in animal production has a growing number of practical benefits, such as in the production of transgenic animals resist to disease, increasing productivity of animals, in the treatment of genetic disorders and in the production of vaccines. This technology will provide various applications in biomedicine that are rather unthinkable without it. Apart from economic constraints, there are concerns of well-being and ethics as introduction of genes from one organism to another may create alteration of the natural genetic balance and lead into undesirable consequences. Notwithstanding the existing limitations, the application of this cutting edge technology to increase livestock productivity could be rewarding in the Ethiopian context. The technology should also be refined in a way to minimize well-being and ethical concerns.

Keywords: Animal production, genetic engineering, gene therapy, vaccines.

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INTRODUCTION

Genetic engineering is the process of taking genes and segments of DNA from one species and putting them into another species, thus breaking the species barrier and artificially modifying the DNA of various species. These procedures are of use to identify, replicate, modify and transfer the genetic material of cells, tissues or complete organisms (Izquierdo, 2001; Karp, 2002). The techniques are generally related to the direct manipulation of DNA oriented to the expression of particular genes (Hugon, 2006).

"Recombinant DNA" (rDNA) is defined as a DNA sequence artificially obtained by combining genetic material from different organisms, as is the case for a plasmid containing a gene of interest (Rossana and Cristina, 2010).

Several developments provided the necessary stimulus for gene manipulation to become a reality. The first major step forward in the ability to chemically modify genes occurred when American biologist Martin Gellert and his colleagues from the National Institutes of Health purified and characterized an enzyme in *Escherichia coli* responsible for the actual joining, or recombining, of separate pieces of DNA. They called their find "DNA-joining enzyme," and this enzyme is now known as DNA

ligase. This enzyme can join two segments of DNA together, a prerequisite for the construction of recombinant molecules, and can be regarded as a sort of molecular glue. A second major step forward in gene modification was the discovery of restriction enzymes, a major milestone in the development of genetic engineering. These enzymes were discovered at approximately the same time as the first DNA ligases by Swiss biologist Werner Arber and his colleagues while they were investigating a phenomenon called host-controlled restriction of bacteriophages. Restriction enzymes are essentially molecular scissors that cut DNA at precisely defined sequences. Such enzymes can be used to produce fragments of DNA that are suitable for joining to other fragments. Thus, by 1970, the basic tools required for the construction of recombinant DNA were available (Desmond, 2008).

Today global life sciences companies are beginning to exploit the new advances in biology in a myriad of ways, laying the economic framework for the Biotech Century. Therefore, the objective of this review is to highlight: (i) the state of the art of genetic engineering, (ii) the potential and approved applications of genetic engineering in biological and medical sciences and (iii) the limitations of its applications.

OVERVIEW OF THE METHODS

Techniques in genetic engineering include; the isolation, cutting and transfer of specific DNA pieces, corresponding to specific genes (Klug and Cummings, 2002).

Vectors

Vectors are DNA molecules used to transfer a gene into a host (microbial, plant, animal) cell; and to provide control elements for replication, selection and expression (Dominic, 2006). Artificial vectors are constructed by cutting and joining DNA molecules from different sources using various restriction endonucleases and DNA ligase (Anil and Neha, 2005). The minimal features of a vector consist of origin of replication, a selection gene (usually an antibiotic resistant gene), and a cloning site to introduce foreign DNA (Cornel, 2007).

Plasmids

Plasmids are circular, double-stranded DNA molecules that can multiply in bacteria independent of the bacterial genome (Figure 1). Because the size of a plasmid is not limited, in principle, you can clone any amount of DNA into them, which makes them wonderful tools in molecular biology (Cornel, 2007).

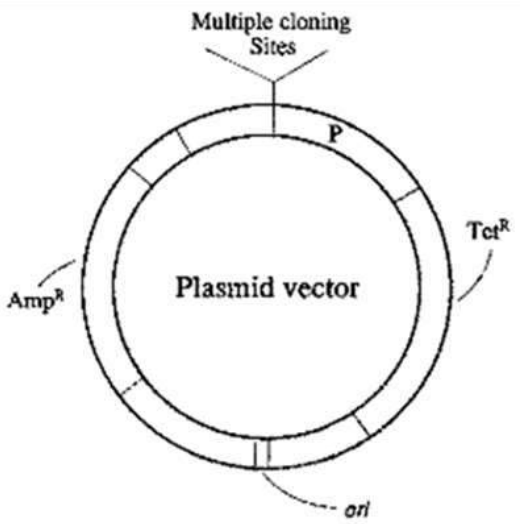

Figure 1. Structural organization of a plasmid vector. Source: Dominic (2006).

Phages

The most important phage in molecular biology is phage lambda, a phage with a 48.5-kb, linear, double-stranded DNA genome, packaged in a protein envelope (Figure 2). This phage infects *E. coli* and replicates in it (Cornel, 2007).

Bacterial artificial chromosomes

Bacterial artificial chromosomes (BACs) are vectors that are made up of fragments up to 300 kb long. They are based on fertility (F) factors, the naturally occurring sex factor plasmid of *E. coli* (Cornel, 2007). Another advantage of BAC over other vectors is its stability in cell culture and ease of manipulation (Warden et al., 2011).

Yeast artificial chromosomes

A yeast artificial chromosome (YAC) is a vector used to clone DNA fragments larger than 100 kb and up to 3000 kb. A YAC is an artificially constructed chromosome that contains a centromere, telomeres and an autonomous replicating sequence (ARS) element required for replication and preservation in yeast cells. ARS elements are thought to act as replication origin (Strachan, 2011). A YAC is built using an initial circular plasmid, which is typically broken into two linear molecules using restriction enzymes; DNA ligase is used to ligate a sequence or gene of interest between two linear molecules, forming a single large linear piece of DNA (Strachan, 2011).

Yeast artificial chromosomes (YACs) are capable of replicating and being selected in common bacterial hosts

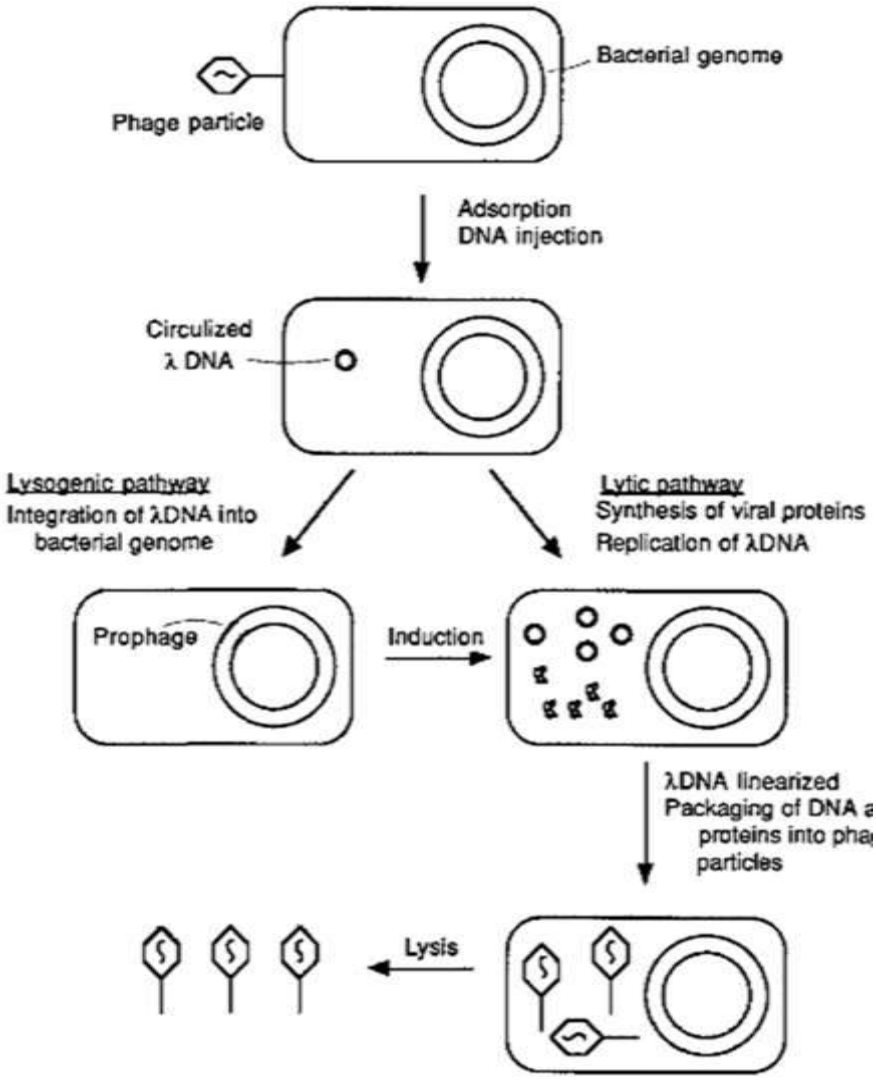

Figure 2. Life cycle of bacteriophage. Source: Dominic (2006).

such as *Escherichia coli*, as well as in the budding yeast *Saccharomyces cerevisiae*. They are of relatively small size (approximately 12 kb) and of circular form when amplified or manipulated in *E. coli*, but rendered linear and of very large size, that is, several hundreds of kilo bases (kb), when introduced as cloning vectors in yeast. Their capacity to accept large DNA inserts enables them to reach the minimum size (150 kb) required for chromosome-like stability and for fidelity of transmission in yeast cells (Carlo and Kresimir, 2002).

Blue script plasmid

The pBluescript II (Figure 3) phagemids (plasmids with a phage origin) are cloning vectors designed to simplify commonly used cloning and sequencing procedures, including the construction of nested deletions for DNA sequencing, generation of RNA transcripts *in vitro* and site-specific mutagenesis and gene mapping. The pBluescript II phagemids have an extensive polylinker with 21 unique restriction enzyme recognition sites. Flanking the polylinker are T7 and T3 RNA polymerase promoters that can be used to synthesize RNA *in vitro*. The choice of promoter used to initiate transcription determines which strand of the insert cloned into the polylinker will be transcribed (Agilent Technologies, 2010).

Gene gun

The gene gun is part of a method called the biolistic (also known as bioballistic) method, and under certain conditions, DNA (or RNA) become “sticky,” adhering to biologically inert particles such as metal atoms (usually

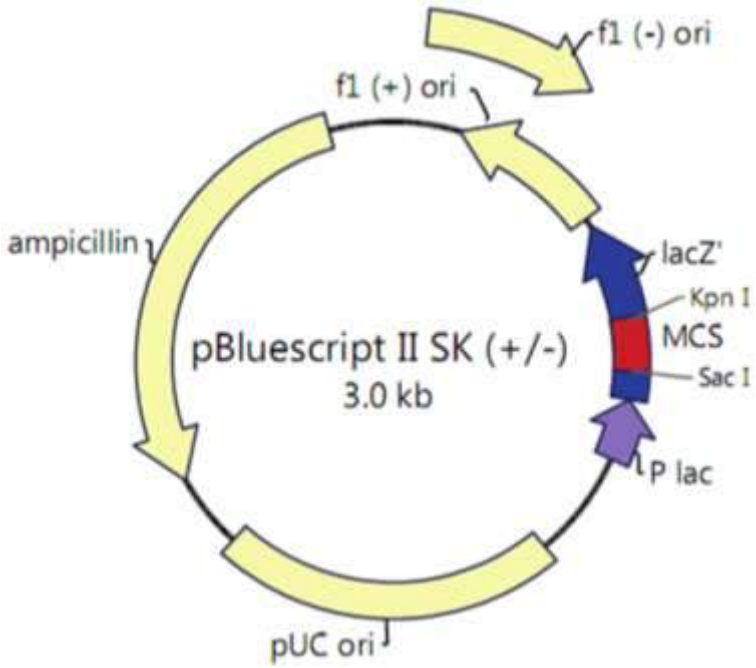

Figure 3. pBluescript II SK (+/–) multiple cloning site region. Source: Agilent technologies (2010).

tungsten or gold). By accelerating this DNA-particle complex in a partial vacuum and placing the target tissue within the acceleration path, DNA is effectively introduced. Uncoated metal particles could also be shot through a solution containing DNA surrounding the cell thus picking up the genetic material and proceeding into the living cell. A device used e.g. for the intraepidermal inoculation of a DNA vaccine. The DNA is coated onto µm-scale gold particles which are internalized via a pressure-based system. This DNA vaccine can be delivered using biolistic bombardment of DNA-coated gold beads by a gene gun similar to that use in the transformation of plant tissues (Dominic, 2006).

Recombination

Foreign DNA is inserted into host cells by combining the foreign DNA with DNA of a vector. If the recombinant DNA gets inside a host cell, it can replicate along with the DNA of the host cell. This means that every time the host bacterium undergoes cell division, each new daughter cell receives a copy of the recombinant DNA, thus amplifying the recombinant DNA with each cell division (Austin Community College, 2006).

In order to use a plasmid to insert foreign DNA into a bacterial cell, two steps are required: First, the foreign DNA must be combined with a plasmid. Second step, a bacterial cell must absorb the recombinant plasmid. For the first step, restriction enzymes and DNA ligase are used. Restriction enzymes are naturally occurring enzymes that cut DNA. Many restriction enzymes are valuable tools in molecular biology. Each restriction enzyme cuts DNA only where a specific sequence of base pairs occurs. The broken bonds between the deoxyribose and phosphate groups that form the “side-rails” of the DNA double helix (the phosphodiester linkages) must also be repaired. DNA ligase is the enzyme that catalyzes this reaction (Austin Community College, 2006).

Site-directed mutagenesis also called site-specific mutagenesis or oligonucleotide-directed (Figure 4) mutagenesis is a molecular biology method that is used to make specific and intentional changes to the DNA sequence of a gene and any gene products. It is used for investigating the structure and biological activity of DNA, RNA, and protein molecules, and for protein engineering (Kilbey, 1995).

APPLICATIONS OF GENETIC ENGINEERING

Disease resistance

Animal biotechnology offers a number of approaches to fight diseases in animals. Firstly, through genetic selection, livestock producers can select for certain traits that are associated with disease resistance and populations of animals that are less vulnerable to diseases could be developed. Secondly, through genetic engineering, breeders can integrate disease resistance genes from new sources, allowing for improved animal

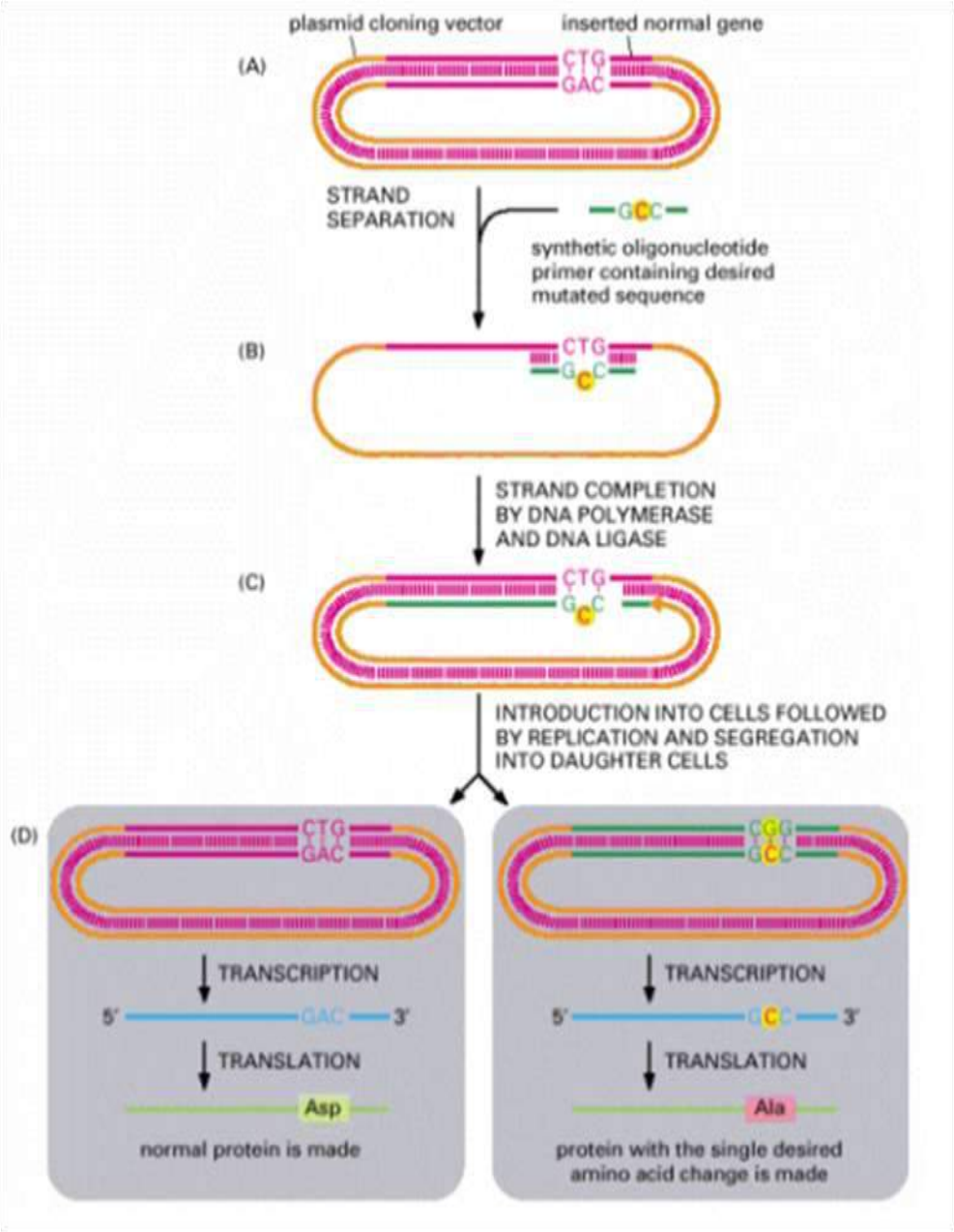

Figure 4. The use of a synthetic oligonucleotide to modify the protein-coding region of a gene by site-directed mutagenesis. Source: Bruce et al. (2002).

health (Table 1). Disease resistance benefits not only livestock producers and their animals, but consumers also benefit as a result of safer animal products in the market , and a reduction in the incidence of human-transmissible diseases such as avian influenza (Alison and Davis, 2009). Increased disease resistance can be achieved by introducing resistance-conferring gene constructs into animals or by depleting a susceptibility gene or locus from the animal (Tables 1 and 2).

Hence gain of function (additive) as well as loss of function (deletive, knockout) gene transfer experiments can be used. Gene transfer experiments are often hampered by the lack of identified major genes or loci responsible for resistance traits (Muller and Brem, 1998).

Increasing meat and milk production

The application of genetic engineering to increase milk and meat is a “value-added” opportunity in animal agriculture as it increases the concentration of existing proteins or producing entirely new proteins (Scott and

Table 1. Extant applications of the production of disease-resistant genetically engineered livestock.

Extant applications	Species	Gene	Proposed approach	References
BSE resistance	Cattle, sheep, goats	Prion	Knockout	Richt et al. (2007)
Mastitis resistance	Cattle	Lysostaphin	Transgene overexpression	Wall (2005)
Mastitis resistance	Cattle	Lactoferrin	Transgene overexpression	Van Berkel (2002)
BSE resistance	Goat	Prion	RNAi transgene	Golding (2006)
Visna virus resistance	Sheep	Visna virus envelope gene	Transgene overexpression	Clements et al. (1994)
Mastitis resistance	Goats	Lysozyme	Transgene overexpression	Mega et al. (2006)
Grass carp haemorrhage virus resistanc	Grass, Carp	Lactoferrin	Transgene overexpression	Zhong et al. (2002)
Bacterial resistance	Channel, Catfish	Cecropin B gene	Transgene overexpression	Dunham and Warr (2002)

Table 2. Envisioned applications of the production of disease-resistant genetically engineered livestock.

Envisioned applications	Species	Gene	Proposed approach	References
Suppressing infectious pathogens	Various	RNA viruses (eg. foot and mouth, fowl plague, swine fever)	RNAi	Whitelaw and Sang (2005)
Corona virus- resistance	Swine	Aminopeptidase N	RNAi /Knockout	Schwegmann-Wessels et al. (2002)
Avian flu resistance	Poultry	Avian influenza	RNAi	Tompkins et al. (2004)
Brucellosis resistance	Cattle	NRAMP1	Transgene overexpression	Barthel et al. (2001)

Mattew, 2011). For example, the presence of 10 to 20% altered casein in milk produced by a transgenic cow could increase proteolysis and thereby promote the faster ripening of cheese. Results of experiments with transgenic mice illustrated the positive effects of adding genes such as the casein gene (Gutiérrez-Adán et al., 1996) or human lysozyme gene (Mega et al., 2006) to the milk protein system (James and Gary, 2000).

The effects of genes encoding growth hormone-releasing factor (GRF) or insulin-like growth factor I (IGF-I) were reported in growth studies in mice and sheep (Murray et al., 1999). In pigs, there are evidences of transgene effects that reduced body fat and increased muscle fiber diameter by increasing IGF-I levels and growth hormone with no serious pathological side effects (Hugon, 2006).

Improving hair and fiber

The quality, color, yield and ease of harvest of hair, wool and fiber for fabric and yarn production have been an area of focus in livestock production. The manipulation of the quality, length, fineness and crimp of wool, hair and fiber from sheep and goats has been examined using transgenic methods (Scott and Mattew, 2011). The objectives aimed to improve sheep for wool production and to modify the properties of the fiber. Because cystein seems to be the limiting amino acid for wool synthesis, the approach is to increase its production through transfer of cystein biosynthesis from bacterial genes to sheep genome (Murray et al., 1999).

Enhancing growth rates and carcass composition

The production of genetically engineered livestock has been instrumental in providing new insights into the mechanisms of gene action governing growth. Using transgenic technology, it is possible to manipulate growth factors, growth factor receptors and growth modulators.

Transgenic sheep and pigs have been used to examine postnatal growth of mammals. Growth hormone (GH) and IGF genes were incorporated and expressed at various levels in genetically engineered animals. Transgenic livestock and fish that contain an exogenous GH gene were produced. Altering the fat or cholesterol composition of carcass is another benefit that can be delivered via genetic engineering. By changing the metabolism or uptake of cholesterol and/or fatty acids, the content of fat

and cholesterol of meats, eggs and cheeses could be lowered. There is a possibility of introducing beneficial fats such as the omega-3 fatty acids from fish or other animals in livestock (Lai et al., 2006). Receptors such as the low-density lipoprotein (LDL) receptor gene and hormones like leptin are also potential targets that would decrease fat and cholesterol in animal products (Scott and Mattew, 2011).

Improving reproductive performance and fecundity

Several genes that may profoundly affect reproductive performance were identified. These included the estrogen receptor (ESR) and the Boroola fecundity (FECB) genes. A specific form of the ESR gene is associated with 1.4 more pigs born per litter than is typical in lines of pigs that do not contain this specific ESR gene type (Rothschild et al., 1994). Introduction of a mutated or polymorphic ESR gene could increase litter size in pigs. A single major gene for fecundity, the FECB gene that allows for increased ovulation rate was identified in Merino sheep (Piper et al., 1985). Each copy of this gene increases ovulation rate by approximately 1.5 ova per cycle (Scott and Mattew, 2011).

Vaccine production

Most conventional vaccines are killed microorganisms, inactivated bacterial toxins or live attenuated organisms. However, because the immune system acts only on a few protective immunogens, most molecular components of killed vaccines are redundant and or can cause adverse effects. Nowadays new technologies offer alternatives to classical vaccines (Sussan and Asa, 1998).

Live genetically modified vaccines

Live genetically modified vaccines could be viruses or bacteria with one or more genes deleted or inactivated, or they can be vaccines carrying a foreign gene from another disease agent, which are referred to as vaccine vectors. Deletion of a gene or genes is to inactivate or attenuate the disease agent. Generally two (double-knockout) or more genes are deleted or inactivated so the vaccine remains stable and cannot revert to a pathogenic agent (Uzzau et al., 2005).

Recombinant inactivated vaccines

Recombinant inactivated vaccines are subunit vaccines containing only part of the whole organism. Subunit vaccines are synthetic peptides that represent the most basic portion of a protein that induces an immune response. Subunit vaccines consist of whole proteins extracted from the disease agent or expressed from cloned genes in the laboratory. Several systems can be used to express recombinant proteins, including expression systems that are cell free or that use whole cells. Whole-cell expression systems include prokaryotic (bacteria-based) systems such as *E. coli*, and eukaryotic (mammalian, avian, insect, or yeast-based) systems. Another type of recombinant subunit vaccines, called virus like particles (VLPs), can be created when one or more cloned genes that represent the structural proteins of a virus are expressed simultaneously and self-assemble into VLPs. These VLPs are immunogenic. Because subunit vaccines do not replicate in the host, they usually are administered with an adjuvant (Mark et al., 2008).

Genetic vaccines

Genetic vaccines are circular pieces of DNA, called plasmids, which contain a foreign gene from a disease agent and a promoter that is used to initiate the expression of the protein from that gene in the target animal (Rodriguez and Whitton, 2000). Plasmids can be maintained in bacteria (usually *E. coli*) and have been designed to accept foreign genes for expression in animals. Recombinant plasmids containing a foreign gene are purified from bacteria, and “naked” DNA is injected directly into an animal, intramuscularly or intradermally (Mark et al., 2008).

In addition to genes coding for immunogenic proteins, genetic vaccines are designed to include different immune-stimulatory genes that trigger different compartments of the immune system, depending on the type of immunity desired. Unique features of DNA vaccines are intrinsic sequences embedded in the DNA, so-called CpG motifs. These unmethylated motifs were shown to act as an adjuvant, stimulating the innate immune responses and enhancing the effectiveness of the vaccine (Mark et al., 2008).

Plant based vaccines

A novel approach for developing subunit vaccines has emerged as a result of the use of plants as hosts-biological bioreactors (Schuyler, 2008). Plant-based vaccines consist of protein subunits. A good candidate antigen must first be identified in order to develop the vaccine. Edible plant derived vaccines take advantage of the ability of some antigens to induce an immune response when delivered orally. Foreign genes from disease agents are inserted into potatoes, soybeans, and corn plants and fed to animals and the expressed proteins from these foreign genes immunized the animals against the disease agent (Streatfield, 2005).

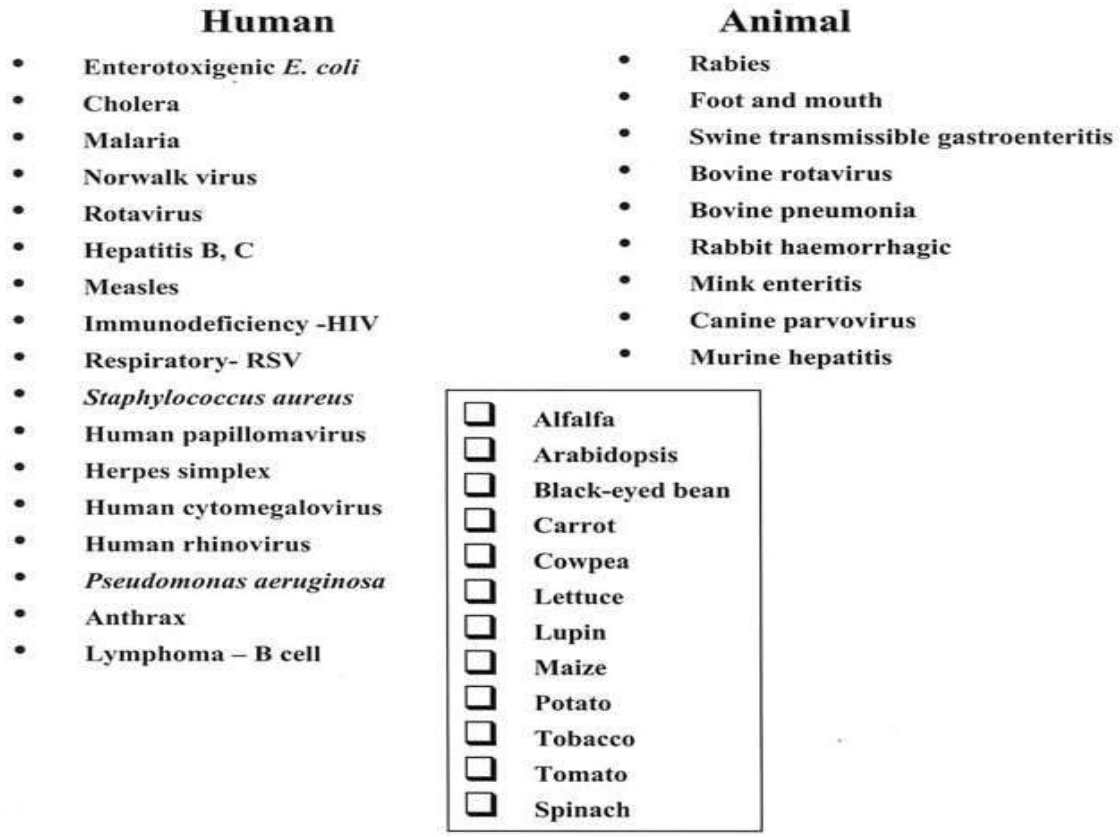

Figure 5. Subunit vaccine candidates against human and animal diseases for possible production in various crops. Source: Schuyler (2008).

Edible plant-based vaccines eliminate the need for disposable injection materials and trained personnel to administer the injections. Moreover, farmed animals do not need to be gathered to receive an injection; an edible product can conveniently be added to their regular feed. Certain types of plant material, such as grains store proteins in a dehydrated and stable condition for years at ambient temperatures, thus allowing for inexpensive storage and distribution. In addition, since plant-based vaccines are subunit vaccines, there tend to be fewer safety concerns than when considering live delivery vehicles (Streatfield, 2005). Today there are several subunit vaccine candidates against human and animal diseases for possible production in crops (Figure 5).

Gene therapy

Gene therapy is fundamentally an attempt to grasp hereditary diseases at their origin. The underlying idea is to repair a mutation in a gene (Cornel, 2007). It is the use of DNA as a pharmaceutical agent to treat disease. It derives its name from the idea that DNA can be used to supplement or alter genes within an individual's cells to a treat disease. The most common form of gene therapy involves using DNA that encodes a functional, therapeutic gene to replace a mutated gene. Other forms involve directly correcting a mutation, or using DNA that encodes a therapeutic protein drug (rather than a natural gene). In gene therapy, DNA that encodes a therapeutic protein is packaged within a "vector", which is used to get the DNA inside cells within the body. Once inside, the DNA becomes expressed by the cell machinery, resulting in the production of therapeutic protein (Sheridan, 2011).

Any form of therapy that involves genetic modification of (particular) cells with the object of correcting a genetic disorder or of treating certain types of infectious disease. Public awareness of gene therapy focuses primarily on the treatment of inherited disorders (such as cystic fibrosis and adenosine deaminase deficiency) but much work has also been carried out on infectious diseases, such as those caused by hepatitis, influenza and human immunodeficiency viruses and by Mycobacterium tuberculosis (Paul, 2008).

Two basic approaches in gene therapy are: (i) the *ex vivo* mode, in which cells are removed from the body, genetically modified *in vitro*, and then returned to the body; (ii) the *in vivo mode*, in which genetic modification is carried out *in situ*, the modifying agent being internalized (Paul, 2008).

PROSPECTS OF GENETIC ENGINEERING IN DEVELOPING COUNTRIES

Socio-economic impacts

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 and the application of biotechnology is largely dictated by commercial considerations and socioeconomic goals. The use of biotechnology to support livestock production is an integral part of viable agriculture in multi-enterprise systems (Madan, 2005).

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 (Madan, 2005).

Impacts on human and animal health

Healthcare research is the most well-known purpose for which animals are genetically engineered. Through animal genetic engineering, scientists have made major breakthroughs in organ transplantation, cancer research, and other areas. Similarities between the genomes of humans and other animals also suggest that future genetic research on animals will yield additional benefits for humans. In the future, kidney, heart, and lung failure patients will likely benefit from animal organ transplants. Xenotransplantation is the procedure of transplanting organs from one species to another. Although xenotransplantation is not new, its use to solve immunological problems such as transplant rejection began recently. Some believed that animal organ transplantation may be able to solve the organ shortage problem (Chad, 2006). The application of modern biotechnology to food production presents new opportunities and challenges for human health and development. Recombinant gene technology, the most well known modern biotechnology, enables plants, animals and microorganisms to be genetically modified (GM) with novel traits beyond what is possible through traditional breeding and selection technologies (WHO, 2005).

The inclusion of novel traits potentially offers increased agricultural productivity, or improved quality and nutritional and processing characteristics, which can contribute directly to enhancing human health and development. From a health perspective, there may also be indirect benefits, such as reduction in agricultural chemical usage, and enhanced farm income, crop sustainability and food security, particularly in developing countries (WHO, 2005).

LIMITATIONS OF GENETIC ENGINEERING

Environmental impacts

Potential risks for the environment include unintended effects on non-target organisms, ecosystems and biodiversity. Insect-resistant GM crops have been developed by expression of a variety of insecticidal toxins from the bacterium *B. thuringiensis (Bt*). Detrimental effects on beneficial insects, or a faster induction of resistant insects (depending on the specific characteristics of the *Bacillus thuringiensis* proteins, expression in pollen and areas of cultivation), have been considered in the area of a number of insect-protected GM crops (Sears et al., 2001).

Health hazards

There are crucial scientific questions concerning the health effects of genetic engineering and genetically engineered organisms. It has been argued that random insertion of genes in may cause genetic and phenotypic instabilities (Ho, 2002). If DNA and proteins from genetically engineered organisms persist in, and are taken up from the mammalian gastrointestinal tract, this could theoretically, ultimately lead to development of chronic disease conditions (Tereje and Jack, 2007).

Religious, cultural and ethical Issues

The current and potential impact of rapid developments in biotechnology to effect new innovations in medicine and drug development, as well as such diverse areas as crime detection, agriculture, pollution control and industrial processes, brings into question how these techniques can be used constructively without damaging the cornerstones of religious ethics, namely respect for human life (Curran and Koszarycz, 2004). Revolutionary innovations in genetic engineering, the decoding of the human genome now make it possible for vegetables in our food chain to bear animal transgenes (Conrad and Harold, 2009).

Public opinion surveys have reported that some people are ethically uncomfortable with the idea of genetically engineering animals. There are two central ethical concerns associated with the genetic engineering of

animals. The first has to do with breaching species barriers or playing God. Proponents of this view suggest that life should not be regarded solely as if it were a chemical product subject to genetic alteration and patentable for economic benefit. The second major ethical concern is that the genetic engineering of animals interferes with the integrity or telos of the animal. Telos is defined as "the set of needs and interests which are genetically based, and environmentally expressed, and which collectively constitute or define the form of life or way of living exhibited by that animal, and whose fulfillment or thwarting matter that animal" (Alison, 2008).

Economic constraints

The requirement of adequate infrastructure is a critical factor for the establishment of biotechnology companies. While research and development budgets for biotechnology research are only beginning to pick up in developing countries, start-up funding for biotechnology companies is still very rare to come by. Financing early stages of business development could be achieved through seed funding, easier access to loans and venture funds (Nyerhovwo, 2004).

Antibiotic resistance

The major concern with the use of antibiotic resistance marker genes is that they will diminish the efficacy of antibiotics in humans and animals. The large presence of antibiotic resistance genes in the environment and soil, as well in the food eaten by animals and humans, could pass the trait of antibiotic resistance rapidly and widely. This can occur through a transfer of antibiotic resistance marker genes to bacteria in the guts of animals or humans, or to bacteria in the environment. Many bacteria have the ability to pick up genes from their surroundings and to pass these genes on to other species of bacteria including antibiotic resistance marker genes. Such genes might eventually find their way into disease-causing bacteria, resulting in antibiotic resistance and therefore making treatment more difficult (Prakash, 2002).

The primary marker genes are either antibiotic resistance or herbicide tolerance. When an herbicide tolerance gene is used as the selectable marker, if herbicide is added to the plant culture media it kills the plant cells that did not incorporate the herbicide tolerance marker gene, while the few cells that did incorporate the gene survive. The same logic applies for the antibiotic resistance marker gene. When an antibiotic is added to the plant culture media, it kills the plant cells that did not incorporate the antibiotic resistance marker gene, and any cells that did incorporate the gene will live (Prakash, 2002).

RECOMBINANT DNA TECHNOLOGY IN ETHIOPIA

Ethiopia is an agrarian country that can have enormous benefit from the applications of biotechnology for increasing its agricultural productivity. The country is at initial stages of research and development in agricultural biotechnology. Livestock related applications include artificial insemination, molecular diagnostics, vaccine production and molecular genetic analysis.

Infrastructure and skills in recombinant DNA and other cutting edge technologies such as proteomics and bioinformatics are not available. Ethiopia has recently given a due emphasis for capacity building in agricultural biotechnology extending from promoting research, development and education in various public institutions to setting up of an independent agricultural biotechnology research center. The constraints holding back progress in agricultural biotechnology are numerous ranging from poor technical and regulatory capacity to lack of appreciation of opportunities provided by agro biotechnology by the public and decision makers (Adane, 2009).

The Ethiopian Ministry of Agriculture is seeking to advance the capacity of various Ethiopian agricultural research institutes in the field of biotechnology. The Ministry requested a set of guidelines that will aid to direct national and international resources in order to establish a platform of technologies in the areas of molecular genetics, cell and tissue culture, and genetic engineering. These technologies are expected to increase productivity and raise the competitive marketability of Ethiopian agricultural products in national and international trade (Eli, 2004).

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REFERENCES

Adane A, 2009. Agricultural biotechnology research and development in Ethiopia. African Journal of Biotechnology, 8:7197.

Agilent Technologies, 2010. pBluescript II Phagemid Vectors. Instruction Manual. pp 1-4.

Alison VE, 2008. Genetically engineered animals: An overview. Department of Animal Science, University of California, California, pp 4-5.

Alison VE, Davis UC, 2009. Potential effects of biotechnology on animal health and well-being. Applications of Animal Biotechnology in Animal Health, Department of Animal Science, University of California, California, pp 1-5.

Anil K, Neha G, 2005 Genetic Engineering. 1st Edition. Nova Science Publisher, Inc, New York, pp 15-16.

Austin Community College, 2006. Laboratory Manual. 12th Edition. Texas, pp 1-6.

Barthel R, Feng J, Piedrahita J, McMurray D, Templeton J, Adams L, 2001. Stable transfection of the bovine NRAMP1 gene into murine

cells: effect on *Brucella abortus* survival. Infection and Immunity, 69:3110-3119.
Bruce A, Alexander J, Julian LMR, Keith R, Peter W, 2002. Molecular Biology of the Cell. 4th Edition. Garland Publishing, New York, pp: 1360-1373.
Carlo V, Kresimir G, 2002. Yeast Artificial Chromosomes. International Centre for Genetic Engineering and Biotechnology, Trieste, pp: 1-3.
Chad W, 2006. Economics and Ethics in the Genetic Engineering of Animals. Harvard J Law Technol, 19:415-424.
Clements J, Wall R, Narayan O, Hauer D, Schoborg R, Sheffer D, Powell A, Carruth L, Zink M, Rexroad C, 1994. Development of transgenic sheep that express the visna virus envelope gene. Virology, 200:370-380.
Conrad G, Harold C, 2009. Acceptable Genes. Religious, Traditional and Genetically Modified Foods. State University of New York Press, Albany, pp 135-140.
Cornel M, 2007. Molecular Biology and Genomics. 1st Edition. Elsevier Inc, San Diego, pp 116-218.
Curran GJ, Koszarycz YJ, 2004. Genetic engineering: Creating an ethical framework. Australian Journal of Theology, 2:1-6.
Desmond STN, 2008. An Introduction to Genetic Engineering. 3rd Editio n. Cambridge University Press, University of the West of Scotland, Paisley, pp 3-240.
Dominic WSW, 2006. The ABCs of Gene Cloning. 2nd Edition. Springer Science+Business Media, Inc, New York, pp 93-180.
Dunham RA, Warr GW, 2002. Enhanced bacterial disease resistance of transgenic channel catfish Ictaluruspunctatus possessing cecropin genes. Marine Biotechnol, 4:338-344.
Eli K, 2004. A feasibility mission for the use of biotechnology in agricultural development. Ministry of foreign affairs center for international cooperation (mashav), pp 3-4.
Golding MC, Long CR, Carmell MA, Hannon GJ, Westhusin ME, 2006. Suppression of prion protein in livestock by RNA interference. Proc Nat Acad Sci United States Am, 103:5285-5290.
Gutiérrez-Adán A, Maga E, Meade H, Shoemaker C, Madrano J, Anderson G, Murray J, 1996. Alteration of physical characteristics of milk from bovine kappa-casein transgenic mice. J Dairy Sci, 79:791-799.
Ho MW, 2002. The best kept secret of GM crops. Witness Statement to ACRE for ACRE open hearing on the criticisms of T25 GM maize risk assessment. Institute of Science in Society, http://www.i-sis.org.uk/secretGMcrops.php.
Hugon HM, 2006. Genetic engineering applications in animal breeding. Elect J Biotechnol, 9:158-162.
Izquierdo RM, 2001. Genetic Engineering. 2nd Edition. Pyramid, Madrid, pp 344.
James DM, Gary BA, 2000. Genetic engineering and cloning may improve milk, livestock production. California Agric, 54:62-63.
Karp G, 2002. Cell and Molecular Biology: concepts and Experiments. 3rd Edition. John Wiley and Sons, New York, Pp 785.
Kilbey BJ, 1995. Charlotte Auerbach (1899-1994)". Genetics, 141:1-5.
Klug WS, Cummings MR, 2002. Concepts of Genetics. 7th Edition. Prentice Hall, New Jersey, Pp 800.
Lai L, Kang J, Li R, Wang J, Witt W, Yong H, Hao Y, Wax D, Murphy C, Rieke A, Samuel M, Linville M, Korte S, Evans R, Starzl T, Prather R, Dai Y, 2006. Generation of cloned transgenic pigs rich in omega-3 fatty acids. Nat Biotechnol, 24:435-436.
Madan ML, 2005. Animal biotechnology: applications and economic implications in developing countries. Revue Scientifique et Technique-Office International Des Epizooties, 24:127-139.
Mark W, Leslie H, Sanjay K, 2008. Vaccine development using recombinant DNA technology. Council for agricultural science and technology. Anim Agric Fut Biotechnol, 7:2-4.
Mega EA, Shoemaker CF, Rowe JD, BonDurant RH, Anderson GB, Murray JD, 2006. Production and processing of milk from transgenic goats expressing human lysozyme in the mammary gland. J Dairy Sci, 89:518-524
Muller M, Brem G, 1998. Transgenic approaches to the increase of disease resistance in farm animals. Revue Scientifique et Technique-Office International Des Epizooties, 17:365-373.
Murray JD, Oberbauer AM, Mcgloughlin MM, 1999. Transgenic animals in agriculture. CABI Publishing, Davis, pp 304.
Nyerhovwo JT, 2004. Fostering biotechnology entrepreneurship in developing countries. Afr J Biotechnol, 3:291-300.
Paul S, 2008. Dictionary of DNA and Genome Technology.1st Edition. Blackwell Publishing, USA, Pp 100.
Piper LE, Bindon BM, Davis GH, 1985. The single inheritance of the high litter size of the Boorola Merino. In: Land, R.B., D.W. Robinson [ed], Genetics of Reproduction in Sheep. Butterworths, London. pp 115-125.
Prakash CS, 2002. Look Mom! No Antibiotic Resistance Marker Genes! Available at http://www.biotechinfo.net/LOOK_MOM.html.
Richt J, Kasinathan P, Hamir A, Castilla J, Sathiyaseelan T, Vargas F, Sathiyaseelan J, Wu H, ,Matsushita H, Koster J, Kato S, Ishida I, Soto C, Robl J, Kuroiwa Y, 2007. Production of cattle lacking prion protein. Nat Biotechnol, 25:132-138.
Rodriguez F, Whitton J, 2000. Enhancing DNA immunization. Virology, 268:233-238.
Rossana D, Cristina G, 2010. Towards the recombinant drug. Eur Mol Biol Lab, 4:34-35.
Rothschild M, Jacobson C, Vaske D, Tuggle C, Short T, Sasaki S, Eckardt G, McLaren D, 1994. A major gene for litter size in pigs. In: Proceedings of the Fifth World Congress Genet Applied to Livestock Production, 21:225-228.
Schuyler SK, 2008. Opportunities and Challenges for Plant-Based Vaccines. Agricultural Biotechnology: Beyond Food and Energy to Health and the Environment, Pp 71-73.
Schwegmann-Wessels C, Zimmer G, Laude H, Enjuanes L, Herrler G, 2002. Binding of transmissible gastroenteritis coronavirus to cell surface sialoglycoproteins. J Virol, 76:6037-6043.
Scott G, Mattew B, 2011. Genetically engineered animals and public health. Compelling Benefits for Health Care, Nutrition, the Environment, and Animal Welfare, Pp 22-30.
Sears MK, Hellmich RL, Stanley-Horn DE, Oberhauser KS, Pleasants JM, Mattila HR, Siegfried BD, Dively GP, 2001. Impact of *Bacillus thuringiensis* corn pollen on monarch butterfly populations: a risk assessment. Proc Nat Acad Sci, 98:11937-11942.
Sheridan C, 2011. Gene therapy finds its niche. Nat Biotechnol, 29:121-128
Strachan T, 2011. Human molecular genetics / Tom Strachan and Andrew Read. 4th Edition. Garland Science, New York, Pp 98-114.
Streatfield SJ, 2005. Plant-based vaccines for animal health. Revue Scientifique et Technique-Office International Des Epizooties, 24:189-199.
Sussan E, Asa M, 1998. The Merck veterinary manual. 8th Edition. Merck and Co. Inc. White House station, New York, Pp 187-1880.
Tereje T, Jack H, 2007. Genetic Engineering and Omitted Health Research: Still No Answers to Ageing Questions. TWN Biotechnology and Biosafety Series, 7:1-14.
Tompkins S, Lo C, Tumpey M, Epstein S, 2004. Protection against lethal influenza virus challenge by RNA interference *in vivo*. Proc Nat Acad Sci United States Am, 101:8682-8686.
Uzzau S, Marogna G, Leori G, Curtiss R, Schianchi G, Stocker B, Rubino S, 2005. Virulence attenuation and live vaccine potential of aroA, crpcdtcya, and plasmid cured mutants of *Salmonella enteric* serovar *Abortus ovis* in mice and sheep . Infect Immun, 73: 4302-4308.
Van Berkel PH, 2002. Large scale production of recombinant human lactoferrin in the milk of transgenic cows. Nat Biotechnol, 20:484-487.
Wall RJ, 2005. Genetically enhanced cows resist intramammary Staphylococcus aureus infection. Nat Biotechnol, 23:445-451.
Warden C, Tang Q, Zhu H, 2011. Herpesvirus BACs: past, present, and future. J Biomed Biotechnol, 27:124595.
Whitelaw CB, Sang HM, 2005. Disease-resistant genetically modified animals. Revue Scientifique et Technique-Office International Des Epizooties, 24:275-283
World Health Organization, 2005. Modern food biotechnology, human health and development: an evidence-based study. Department of Food Safety, Zoonoses and Foodborne Diseases, 20 Avenue Appia, 1211 Geneva 27, Switzerland. Pp 1-23.
Zhong JY, Wang YP, Zhu ZY, 2002. Introduction of the human lactoferrin gene into grass carp (*Ctenopharyngodon idellus*) to increase resistance against GCH virus. Aquaculture, 214:93-101.