Livestock improvement using biotechnology: world and Bangladesh perspective

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Abstract

Genetic engineering is a multidisciplinary subject that covers a wide array of techniques ranging from traditional biotechnology to recombinant DNA technology. There remain different ways of gene manipulation such as using retroviral vector, DNA microinjection, transposon or intracytoplasmic sperm injection. This review focuses on the uses of these techniques in livestock improvement. Several perspectives of worldwide genetic engineering in animal model have been outlined in this review. Improving meat, milk, wool, feed; biopharming; managing excreta better and engineering rumen microbiome are some of the buzzwords in the field of modern day biotechnology. Bangladesh being a developing country, albeit inadequacies, is also focusing on these techniques in a limited way. The review discusses several biotech perspectives taken up by Bangladesh in this regard. The development in feed improvement section has been quite note-worthy. Using locally available plant materials and simple fermentation technology, there has been at least six highly used feed combinations invented by local research institutes in last 10 years. In addition, native solutions to animal diseases have been formulated- at least four different vaccines have become widely popular against animal pathogens. In overall analysis, the review covers applications of biotechnology in animal model juxtaposing Bangladesh and world status of it and proposes an action plan for developing countries for desired improvement.

Key words: animal biotechnology; genetic engineering; biopharming; ruminal microbiota; disease resistance; feed improvement

1.0 Introduction

There has been a constant increase in the demand of livestock and livestock related products worldwide. However, today the world production merely meets the demand to a significant extent. No wonder this hs made scientists to try to improve livestock and livestock associated derivatives. With genetic manipulation and related technologies gaining prominence more and more, research interests to improve livestock using genetic engineering has become a buzzword today; day by day more focuses are being put in this regard (Onteru *et al.* 2010).

Genetic engineering refers to the direct manipulation of an organism's genome using modern DNA technology that involves integration of foreign DNA or synthetic genes into the organism of interest, necessary gene manipulation for desired output and a desired change in genotype or phenotype. Genetic engineering has become a popular choice worldwide as it can be used to break the species barrier, can bring about a change in gene expression and can give rise to 'tailor made' animals for the purpose of Biopharming (Gordon *et al.* 1980).

Genetic engineering and biotechnology is a two-forked discipline; it includes traditional and modern forms. Traditional biotechnology refers to early forms of using living organisms to produce new commodities or modify existing ones. It includes techniques such as selective breeding, fermentation and hybrid animal formation. On the other hand Genetic engineering falls within the ambit of modern biotechnology (Robl *et al.* 2007).

Domesticated animals raised in an agricultural setting to produce commodities such as food, fiber and labor are called livestock as a whole. Although the term is often loosely used to include poultry and fish, more often than not it is used to mean animal husbandry, excluding fisheries and poultry out of its purview. However, for the purpose of generalized discussion this report also focuses briefly on poultry and fisheries aspects.

2.0 Methods in genetic engineering

Genetic engineering is a multistep process. Critical steps involved in the production of transgenic farm animals are often complex and requires careful planning. The genetic engineering workflow starts with an initial identification of the gene of interest which would consequently be cloned. A suitable gene construct would then be produced form it; the gene construct has to be cloned again and readied for transfection. After successful tranfection has been proven expression of the gene of interest would next has to be confirmed. Subsequently inheritance of the gene in the following generation would have to be seen to confirm the stability of the construct. Finally using selective breeding a transgenic line has to be established. These are the processes followed regularly as a means of animal gene manipulation.

The first successful gene transfer method in animals (mouse) was based on the microinjection of foreign DNA into zygotic pronuclei. However, microinjection has several major shortcomings including low efficiency, random integration and variable expression patterns which mainly reflect the site of integration. Research has focused on the development of alternate methodologies for improving the efficiency and reducing the cost of generating transgenic livestock. These include sperm mediated DNA transfer (Baccetti & Spadafora 2000, Chan *et al.* 2000, Khoo 2000, Lauria & Gandolfi 1993, Lavitrano *et al.* 2003, Lavitrano *et al.* 1997, Maione *et al.* 1998, Nakanishi & Iritani 1993, Shamila & Mathavan 1998, Smith & Spadafora 2005), intracytoplasmic injection (ICSI) of sperm heads carrying foreign DNA injection or infection of oocytes (Cai *et al.* 2011, Li *et al.* 2012, Lu *et al.* 2011, Umeyama *et al.* 2012, Yu *et al.* 2011) and/or embryos by different types of viral vectors (Ishii *et al.* 2004, Kimura *et al.* 1994), RNA interference technology (RNAi) (Wise *et al.* 2008) and the use of somatic cell nuclear transfer (SCNT) (Mir *et al.* 2005). To date, somatic cell nuclear transfer, which has been successful in 13 species, holds the greatest promise for significant improvements in the generation of transgenic livestock. Furthermore, there are some common ways of manipulating the animal genome.

2.1 Retroviral Vector Method

Of the various gene transfer method, the retroviral vectors has the advantage of being an effective means of integrating_ the transgene into the genome of a recipient cell. However, these, vectors can transfer only small pieces (~ 8 kilobases) of DNA, which, because of the size constraint, may lack essential adjacent sequences for regulating the expression of the transgene (Squire *et al.* 1989). Major drawback of this method is that the retrovirus may well revert to a pathogenic form to cause diseases such as cancer etc.

2.2 DNA Microinjection Method

Because of the disadvantages of the retroviral vector method, microinjection of DNA is currently the preferred method for producing transgenic mice (Gordon *et al.* 1980, Murphy 1993, Wawra 1988). This procedure is performed via the following steps **(Figure 1)**-

- The number of available fertilized eggs that are to be inoculated by microinjection is increased by stimulating donor females to superovulate. They are given an initial injection of pregnant mare's serum and another injection, about 48 hours later, of human chorionic gonadotropin. Superovulated mother produces 35 eggs instead of the normal 5 to 10.
- The superovulated females are mated and then sacrificed. The fertilized eggs are flushed from their oviducts.
- Microinjection of the fertilized egg usually occurs immediately after their collection. The microinjected transgene construct is often in linear form and free of prokaryotic vector DNA sequences

2.3 Use of Transposon

Transposons are short genomic DNA regions which are replicated and randomly integrated into the same genome. The number of a given transposon is thus increasing until the cell blocks this phenomenon to protect itself from a degradation of its genes. Foreign genes can be introduced into transposon in vitro. The recombinant transposons may then be microinjected into one day old embryos. The foreign gene becomes integrated into the embryos with a yield of about 1%. All the transgenic insects are being generated by using transposons as vectors. (Peng *et al.*2010). Transposons are efficient tools but they can harbour no more than 2–3 kb of foreign DNA.

2.4 Use of ICSI (Intracytoplasmic Sperm Injection)

More than a decade ago, it was shown that sperm, incubated in the presence of DNA before being used for fertilization, was able to transfer the foreign gene into the oocyte and generate transgenic mice. This method appeared difficult to use due to a frequent degradation of DNA (Kolbe & Holtz 1999, Yong *et al.* 2003). Transgenic mice and rabbits were obtained by incubating sperm with DNA in the presence of DMSO (dimethylsuphoxide) and by using conventionnal in vitro fertilization (Geraedts & Gianaroli 2012). The method has been greatly improved, mainly by using ICSI. This technique, which consists of injecting sperm into the cytoplasm of oocytes, is currently used for in vitro fertilization in humans. To transfer genes, sperms from which plasma membrane have been damaged by freezing and thawing were incubated in the presence of the gene of interest and further used for fertilization by ICSI. This method has proved efficient in mice . Transposon use and ICSI may be combined to increase the yield of transgenesis (Biggers 2012, Fang *et al.* 2012). ICSI is therefore an excellent method to generate transgenic animals on condition that ICSI is possible in the considered species. One advantage of ICSI is that long fragments of DNA may be used to transfer the gene of interest. Another advantage is that foreign DNA is integrated at the first cell stage of embryos.

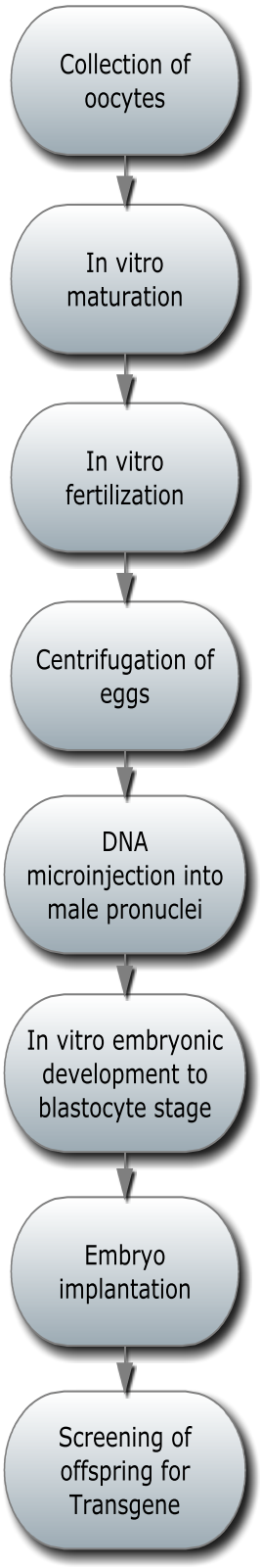

Figure 1: Genetic manipulation in the animal embryo. Eggs are collected and fertilized in vito using a microinjection. The embryo then can be transplanted into a foster mother.

3.0 Current trends: biotechnology in animal model

3.1 Technologies without gene manipulation

Artificial insemination (AI): AI is the process by which sperm is placed into the reproductive tract of a female for the purpose of impregnating the female by using means other than sexual intercourse or natural insemination (Long 2008, Robertson 1954).

Embryo Transfer (ET): In ET, a donor cow of superior breeding is chemically induced to superovulate. The eggs are then fertilized within the donor; the embryo develops and is then removed and implanted in a recipient cow. Between removal and implantation, embryos may be frozen for safekeeping. Because of the relatively high costs, ET is used mostly within registered cowherds (Chen & Wrathall 1989, Stringfellow *et al.* 1991, Waters 1981).

In Vitro Fertilization: With in vitro fertilization (IVF), a technician removes unfertilized eggs (oocytes) from the donor cow's ovaries, usually recovering 6-8 useable oocytes. The oocytes mature in an incubator and are fertilized with sperm. The resulting zygotes incubate and develop in the laboratory before being placed into the recipient cow. While IVF can produce many fertilized embryos, the added expense of ET makes the procedure prohibitive in most cases (Bregulla *et al.* 1970, Motli & Fulka 1974, Yanagimachi 1972).

Sexing Embryos: The dairy industry prefers heifers and the beef industry prefers bulls. Embryo sexing methods in cattle have been developed using a bovine Y-chromosome probe. Technicians remove a few cells from the embryo and assess the DNA in these cells for the presence of a Y-chromosome. Presence of an Y-chromosome determines the embryo is male. Research is also developing in sperm sexing technology (Hare 1986, Rao & Totey 1992, van Vliet *et al.* 1989).

3.2 Technologies with Gene manipulations

Meat and carcass composition: Improvement in the following fields have been the interest of research for r-DNA technology-

- Decreased milk fat composition/ lean meat
- Make meat less chewy/ tenderization of meat
- Improve meat protein content/increased level of casein content

Milk improvement: Current research techniques involve improvement in Milk content are-

- Improve meat protein content/increased level of casein content
- Increased calcium content
- Increased phosphorus content
- Low, mid and high fat content milk
- Decreased milk urea nitrogen (MUN)
- Increased efficiency of cheese production

Wool production: Good quality wool is of high interest. Sheep are the prime target for producing wool. Research interest here lies in-

- High expression of keratin gene

Feed production: Feed improvement basically involves grass and grass-like crop improvement for producing better food for goat, sheep, cow and also poultry and fisheries feed. Currrent research interests lies in-

- Producing cheap feed
- Diversification of feed source
- Recombinant growth hormone for fattening

Improved resistance against disease: Viral, bacterial and fungi attack on livestock, poultry and fisheries causes drastic fall in milk, meat, egg and fish production.

- Vaccines produced by r-DNA technology to combat infectious disease
- Disease diagnosis tools/sensors
- Increased viral resistance
- Resistant insects to combat pathogenic counterpart: recombinant biocontrol organism

Excreta management: Excreta management has been most successful in pigs. The recombinant pigs that can degrade phytate has been produced and hence it excretes less phosphate in feces making it safer to dispose

- Decreased phosphate in excreta of 'Enviro' pigs

Rumen engineering: Ruminants such as cow, goat, and sheep have characteristic bacterial fauna in its rumen which helps it to digest foods properly. Ruminal bacteria have been transformed with special plasmids (such as pBHerm, pUC118) that help the ruminants degrade unnatural feed sources. Research interests currently lie in-

- Protection from poisonous feed component
- Increased efficiency in energy conversion
- Increased feed diversity

Transgenesis in diverse organisms: Sheep, Goats and pigs: Sheep, Goat and pigs although do not produce much milk and not suitable to produce products in mammary gland as a result, still hold major import. Current research strategies are-

- Xenotransplantation: animal organs with expressed complement inhibitory protein to inhibit hyperacute rejection mediated by complement.
- Whole Blood replacement or hemoglobin replacement
- β-globin promoter for overproduction of desired gene

Fish: Fishes are good model for transgenesis as their egg development occur outside the ovary. This frees researchers with the sophisticated implantation process into foster mother ovary. Current research strategies are-

- Enhanced growth: e.g. Chinook salmon growth hormone gene was integrated to Atlantic salmon
- Cold tolerant fish: Antifreeze gene from Tilapia was introduced in atlantic salmon.
- Changing feeding layer of fishes: Conversion of carnivore into herbivores
- Improved feed for fish: Cheap fish feed.
- Improved resistance to fish: e.g. Lysozyme gene integration into Atlantic salmon, silk moth antimicrobial protein cecropin gene was integrated to Catfish

Egg production in poultry: Egg production can be increased to a great extent by disease resistance and the steps mentioned before. However some novel techniques are also been formulated in this aspect such as-

- Altered sex ratio in favor of chicken farming
- Two active ovary instead of one.

Biopharming: "Biopharming" (Bio=life + Pharm=pharmaceuticals + Farming=producing in host) means producing unnatural products in the animal host by gene manipulation and integration. This process uses the livestock as bioreactors to produce pharmaceuticals and other essential products.

- Increased vaccine production
- Increased hormone and enzyme production
- Increased pharmaceuticals and other beneficial products
- Xenotransplantation
- Monoclonal antibody production

Animals as model organism: Animals make up good model organism. Mammals such as rodents have been scientist's favorite for some time. However new discoveries have started to prove that pigs and cattles also make up good model organisms. Researches are going on –

- Efficient Alzheimer model
- Effective HIV model
- Effective Cystic fibrosis model

Transgenic pets: Apart from livestock transgenic pets are also becoming popular. Research interests here have been varied such as-

- Ornamental fish
- Fluorescent pets

Table 1 summarizes some of the information regarding transgenic gene sources and uses.

3.2.1 Meat and carcass composition

Transgenic pigs bearing a hMT-pGH and human insulin-like growth factor-I gene (hIGF-I) construct (human metallothionein promoter driving the porcine growth hormone gene) showed significant improvement in economically important traits including growth rate, feed conversion and body composition (muscle/fat ratio) without the pathological phenotype seen with earlier GH constructs (Pursel *et al.* 1989)

An important step towards the production of more healthful pork products was made by creating pigs with a desaturase gene, derived either from spinach or from Caenorhabditis elegans, which increases the non-saturated fatty acid content in the skeletal muscles of these animals (Kues & Niemann 2004); (Saeki *et al.* 2004).

Table 1: Transgenic animal production for different purposes

Purpose	***Animal model***	***Transgenic gene and source***
Faster growth	*Cattle sheep, rabbit, swine*	*Growth hormone from human, bovine, chicken, porcine, mouse*
Altered milk composition	*Cattle*	*Extra copies of casein gene or disruption of lactoglobulin gene; cow*
'Biosteel' production in milk	*Goat*	*Spider*
Reduced phosphorus in swine feces	*Swine*	*Phytase gene from bacteria*
Increased wool production	*Sheep*	*Cysteine synthesis gene from bacteria* *Growth factor from sheep*
Disease production	*Swine, sheep, rabbit*	*Monoclonal antibody using mouse* *Viral envelop genes cultured in sheep*
Xenotransplantation	*Swine*	*CD55 (DAF or Decay Accelerating Factor) and CD59 from human*

The prevalence of the recessive halothane gene, associated with increased stress sensitivity, is higher in breeds with a higher lean meat content. Stress sensitivity results in a higher prevalence of PSE (Pale, Soft, Exudative) meat (McLoughlin & Mothersill 1976, Richards & Smaje 1974). The dominant RN-gene, occurring in Hampshire pure and cross-breds, results in an increased muscle glycogen content, a low ultimate pH and reduced cooking yields. Both porcine somatotropin (pST) and chromium may prove effective tools for improvement of carcass composition in pigs.

3.2.2 Milk composition

The physicochemical properties of milk are mainly due to the ratio of casein variants, making these a prime target for the improvement of milk composition. Dairy production is an attractive field for targeted genetic modification (Baldassarre *et al.* 2008) and it is possible to produce milk with a modified lipid composition by modulation of the enzymes involved in lipid metabolism and to increase curd and cheese yield by enhancing expression of the casein gene family in the mammary gland. The bovine casein ratio has already been altered by the over-expression of beta- and kappa-casein, demonstrating the potential of transgenic technology for improving the economic value of bovine milk (Maga *et al.* 1998, McKee *et al.* 1998, Young *et al.* 1998). It should also be possible to create 'hypoallergenic' milk by knocking out or knocking down the β-lactoglobulin gene. One could envision the production of enhanced 'infant milk' containing human lactoferrin or the production of milk which resists bacterial contamination by expressing lysozyme, the antibacterial component of egg white and human tears (Bleck *et al.* 1998, Hiripi *et al.* 2000). To generate lactose-free milk, a knockout or knockdown at the α-lactalbumin locus would suppress this key step in milk sugar synthesis. Lactose reduced or lactose-free milk would render dairy products suitable for consumption by the large proportion of the world's adult population who do not produce an active intestinal lactase. However this is achieved not without some side-effects (Stinnakre *et al.* 1994).

In the pig, increased transgenic expression of a bovine lactalbumin construct in the mammary gland resulted in increased lactose content and increased milk production which resulted in improved survival and development of the piglets (Stinnakre *et al.* 1994).

3.2.3 Wool production

Transgenic sheep carrying a keratin-IGF-I construct expressed in their skin produced 6.2% more clear fleece than non-transgenic controls and no adverse effects on health or reproduction were observed (Damak *et al.* 1996a, Damak *et al.* 1996b). Similar efforts to alter wool production by transgenic modification of the cystein pathway have met with more limited success, although it is known that cystein is the rate limiting biochemical factor for wool growth (Ward & Armstrong 2000).

3.2.4 Feed improvement

The two crops used in European animal feed that are most likely to be genetically modified are soya and maize, and almost all is imported. Imported soya and maize by-products account for about 20 per cent of raw materials used by UK feed manufacturers and farmers. The table below shows the worldwide percentages of soya, maize, cotton and oilseed rape crops that are GM.

Soya: GM soya is widely grown in the USA and Argentina; Brazil is still the main source of non-GM soya, although the Brazilian government legalised the cultivation of GM soya in March 2005 (Burns *et al.* 2004).

Maize: Spain grows GM fodder maize in any significant quantity (Williams 2008). Derivatives such as maize gluten, a by-product of the alcohol and starch processing industries, are largely imported from the USA, where there is little segregation of GM and non-GM maize (Ryffel 2011).

3.2.5 Improved resistance against disease

Transgenic strategies to enhance disease resistance include the transfer of major histocompatibility complex (MHC) genes, T cell receptor genes, immunoglobulin genes, genes that affect lymphokines, or specific disease resistance genes (Müller and Brem 1991). A prominent example for a specific disease resistance gene is the murine Mx-gene. Production of the Mx1-protein is induced by interferon. This was discovered in inbred mouse strains that were resistant to influenza viruses (Staeheli 1991). Microinjection of an interferonand virus-inducible Mx-construct into porcine zygotes resulted in two transgenic pig lines which expressed the Mx-mRNA; but no Mx protein was detected (Müller et al. 1992). The bovine MxI gene was identified and shown to confer antiviral activity when transfected into in Vero cells (Baise et al. 2004). Transgenic constructs bearing the immunoglobulin-A (IgA) gene have been successfully introduced into pigs, sheep and mice in an attempt to increase resistance against infections (Weidle *et al.* 1991).

Attempts to increase ovine resistance to Visna virus infection by transgenic production of Visna envelope protein have been reported (1994, Zentilin *et al.* 1994).

Passive immunity has been induced against an economically important porcine disease in a transgenic mouse model (Castilla *et al.* 1998). These transgenic mice secrete a recombinant antibody in their milk that neutralized the corona virus responsible for transmissible gastroenteritis (TGEV) and this conferred resistance to TGEV. Knockout of the prion protein is the only secure way to prevent infection and transmission of spongiform encephalopathies including scrapie and BSE (Weissmann 2002). It was possible to knock out the ovine prion locus; however, the cloned lambs carrying the knockout locus died shortly after birth (Denning 2001). On the other hand, cloned cattle with a knockout for the prion locus have been successfully produced and indeed show clear evidence of resistance to BSE infection (Richt et al. 2007). Transgenic animals with modified prion genes will be an appropriate model for studying the development of spongiform encephalopathies in humans.

Transgenic Approaches to Increased Disease Resistance in the Mammary Gland: The level of anti-microbial peptides (lysozyme and lactoferrin) in human milk is many times higher than in bovine milk and transgenic expression of the human lysozyme gene in mice causes a significant reduction in bacterial contamination and a reduced frequency of mammary gland infections (Maga *et al.* 1995, Maga & Murray 1995). Lactoferrin has bactericidal and bacteriostatic effects in addition to being the main source of iron in milk (Krimpenfort *et al.* 1991, Platenburg *et al.* 1994) and was associated with an increased resistance against mammary gland diseases (van Berkel *et al.* 2002). Similarly, lysostaphin was shown to confer specific resistance against mastitis caused by Staphylococcus aureus. Mastitis resistant cows have been produced by expressing a lysostaphin gene construct in the mammary gland (Pitkala *et al.* 2005).

3.2.6 Excreta management

Phytase transgenic pigs have been developed to address the problem of manure-related environmental pollution. These pigs carry a bacterial phytase gene under transcriptional control of a salivary gland specific promoter, which allows the pigs to digest plant phytate. Without the bacterial enzyme, phytate passes through the animal undigested and pollutes the environment with phosphorus if uncontained. With the bacterial enzyme fecal phosphorus output was reduced up to 75% (Golovan *et al.* 2001). These environmentally friendly pigs may be used for commercial production in Canada within the next few years. The pig is called **Enviropig.**

The excreta diposed in the lake water causes huge algal bloom which pollutes reservoir water and a marked reduction in dissolved oxygen in the environment. This leads to fish and other aquatic species death and long time pollution problem. The benefits of the enviropig if commercialized include reduced feed cost and reduced phosphorus pollution as compared to the raising of ordinary pigs.

3.2.7 Rumen engineering

A ruminant is any hoofed animal that digests its food in two steps-

a) By eating the raw material and regurgitating a semi digested form known as cud

b) Then eating the cud, a process called ruminating

Examples are: cattle, goat, sheep, camel, giraffe, buffalo, dear etc.

Fungi: They are known only for about 20 years and usually found low in number. Fungi digest recalcitrant fiber.

Cellulolytic bacteria (fiber digesters): They digest cellulose under pH 6-7 by utilizing N in form of NH_3. They also require S for synthesis of sulfur-containing amino acids (cysteine and methionine) and produce acetate, propionate, little butyrate, CO_2. Cellulolytic bacteria predominate from roughage diets (Chaucheyras-Durand & Fonty 2001, Dehority 1968, Dehority *et al.* 1967, Miron *et al.* 2001, Russell 1985, Russell *et al.* 2009, Shane *et al.* 1969).

Amylolytic bacteria (starch, sugar digesters): They digest starch under pH 5-6 by utilizing N as NH_3 or peptides. They also produce propionate, butyrate and lactate predominate from grain diets. Rapid change to grain diet causes lactic acidosis (rapidly decreases pH) (Laukova *et al.* 1988, Marounek & Bartos 1986, 1987, Therion *et al.* 1982, Walker & Hope 1964).

Methane-producing bacteria: They produce methane (CH_4) by which is utilized by microbes for energy.

Microbial degradation of antinutritional factors that has been tried successfully in the are-

Tannins Toxic Non-protein amino acids: Tannase in food, beverages, in preparation of instant tea and as clarifier in fruit juices and beer; Oxalates; Fluoroacetate; Pyrrolizidine; Phytase: Phytase are used as feed additives in monogastric's foods to increase phosphate utilisation.; *Lactobacillus* species for disease treatment as probiotics (Demeyer & Henderickx 1964, Demeyer & Henderickx 1967, Sauer *et al.* 1977, Sauer & Teather 1987).

3.2.8 Transgenesis in diverse organisms

Sheep, Goats, and Pigs

These studies show that no matter how appealing a concept experience with the growth hormone transgene in pigs, improved wool production due to enhanced growth of fleece in transgenic sheep was achieved by the overexpression of a construct that consisted of a mouse ultra-high sulfur keratin promoter driving a sheep insulin-like growth factor 1 c-DNA without any apparent negative effects on the health of the animals. **Table 2** summarizes some of the examples of transgenesis (Bawden *et al.* 1999, Poggiali *et al.* 2002).

Table 2: Transgenesis in different organisms

Transgene	***Promoter***	***Transgenic Species***
Longer-acting tissue plasminogen	*Whey acidic protien*	*Goat*
α-Antitrypsin	*β- Lactoglobulin*	*Sheep*
Clotting factor IX	*β- Lactoglobulin*	*Sheep*
Soluble CD4 protein	*Whey acidic protien*	*Mouse*
Lactoferrin	*αs1- Casein*	*Cattle*
Urokinase	*αs1- Casein*	*Mouse*
CFTR	*β- Casein*	*Mouse*
Interleukin-2	*β- Casein*	*Rabbit*

As well, there have been successful experimental results with transgenic pigs. For example, with a construct that consisted of the regulatory region from the human β -globin gene joined to two human a 1-globin genes and one human β^A-globin gene, healthy transgenic pigs that expressed human hemoglobin in their blood cells were produced.

Transgenic Birds: Avian researchers have also suggested that the egg, with its high protein content, could be used as a source for pharmaceutical proteins. By analogy to the mammary gland of sheep, goats, and cows, the expression of a transgene in the cells of the reproductive tract *of* a hen that normally secretes large amounts of ovalbumin could lead to the accumulation of a transgene-derived protein that becomes encased in the eggshell. The pharmaceutical protein could then be fractionated from these natural storage cases (Mochii 1995).

Transgenic Fish: As natural fisheries become exhausted, production of this worldwide food resource will depend more heavily on aquaculture. Unlike mammalian embryogenesis, fish egg development is external; hence, there is no need for an implantation procedure. Development can occur in temperature-regulated holding tanks (Chen & Powers 1990, Lin *et al.* 2010, Marris 2010, Nam *et al.* 2000, Ozato *et al.* 1986, Stokstad 2002).

In one study, a transgene consisting of the promoter region from the antifreeze protein gene of the fish called ocean pout, the growth hormone cDNA from salmon, and the termination-polyadenylation signals from the 3' end of the

antifreeze protein gene from the ocean pout was injected into eggs of Atlantic salmon. In general, the transgenic salmon were larger and grew faster than the nontransgenic controls (Gong *et al.* 1991).

3.2.9 Biopharming

Pharmaceutical Production in the Mammary Gland of Transgenic Animals: Gene 'pharming' entails the production of recombinant pharmaceutically active human proteins in the mammary gland or blood of transgenic animals. This technology overcomes the limitations of conventional and recombinant DNA based production systems (Pollock *et al.* 1999, Rudolph 1999) and has advanced to the stage of commercial application (Dyck *et al.* 2003, Rudolph 1999, Ziomek 1998).

Several products derived from the mammary glands of transgenic goats and sheep have progressed to advanced clinical trials (Herrick *et al.* 2006). Phase III trials for antithrombin III (ATIII) (ATryn® from GTC-Biotherapeutics, USA), produced in the mammary gland of transgenic goats, have been completed and the recombinant product was approved as drug by the European Medicines Agency (EMEA) in August 2006.

Antibody Production in Transgenic Animals: Numerous monoclonal antibodies are being produced in the mammary gland of transgenic goats and cloned transgenic cattle have been created which produce a recombinant bi-specific antibody in their blood (Grosse-Hovest *et al.* 2004, Kuroiwa *et al.* 2002, Robl *et al.* 2007) .

Polyclonal Antibodies: Therapeutic monoclonals from whatever source all share the feature that a single antibody binds only one epitope of an antigen whereas polyclonal antibody binds only one type of antigen. Kirin Pharma have created transchromosomic cattle using a human artificial chromosome vector containing the entire un-rearranged sequences of the human immunoglobulin G heavy-chain and lambda light-chain loci (Ren *et al.* 2008).The loci were functional, but transmission of the artificial chromosome through the germ line and stable maintenance over generations may prove difficult. The same researchers have also reported targeted inactivation of the endogenous bovine immunoglobulin-μ heavy chain gene and the. It remains to be seen whether herds of large animals capable of producing human polyclonal antibodies can be raised and bred.

Biopharming in chicken: Currently there are no commercial biopharm operations using chickens; however, scientists have worked with chickens in order to express new enzymes in eggs, albeit in small quantities (Bonifer *et al.* 1996, Salter *et al.* 1987). There are several advantages to using chickens for transgenic operations. Firstly, there are well-established methods for extracting the protein from eggs, and some scientists predict the process will be much the same for transgenic eggs (Etches & Verrinder Gibbins 1997, Pain *et al.* 1999, Volkova *et al.* 2006). Half of the protein in egg white, ovalbumin, can be used to produce therapeutic recombinant proteins (Lillico *et al.* 2007). Secondly, reproduction times are shorter and reproduction rates are higher than those of the mammals discussed above.

Blood Replacement: Functional human hemoglobin has been produced in transgenic swine (Swanson 1992). Alternate approaches to produce human blood substitutes have focused on linking hemoglobin to the superoxide-dismutase system ((D'Agnillo & Chang 1998).

Xenotransplantation of Porcine Organs to Human Patients: To close the growing gap between demand and availability of appropriate organs, transplant surgeons are now considering the use of xenografts from domesticated pigs (Terblanche *et al.* 1970). The two strategies that have been successfully explored for long term suppression of the hyper acute rejection of porcine xenografts are: i) transgenic synthesis of human proteins regulating complement activity (RCAs) in the donor organ (Carrington *et al.* 1995, Rosengard *et al.* 1995, Yannoutsos *et al.* 1995) and ii) inactivation of the genes producing antigenic structures on the surface of the donor organ, e.g. the α-gal-epitope. Prolonged survival of xenotransplanted porcine organs where the 1,3-α-galactosyltransferase (α-gal) gene has been knocked out has been demonstrated. Survival rates of up to six months have been achieved with transplanted porcine hearts (Kuwaki *et al.* 2005) and survival of up to three months has been obtained with kidneys transplanted from α-gal knockout pigs to baboons (Mezrich *et al.* 2005, Yamada 2005). Recently produced triple transgenic pigs expressing either human thrombomodulin (hTM) or human heme oxygenase-1 (hHO-1) on top of one or two RCAs to suppress both HAR and the later stage coagulatory disorders showed porcine-to-primate xenotransplantation (Hammer 2001). A particularly promising strategy for achieving long-term xenograft survival is to induce tolerance by creating permanent chimerism in the recipient by intraportal injection of embryonic stem cells (Fandrich *et al.* 2002a, Fandrich *et al.* 2002b) or by co-transplantation of vascularized thymic tissue. Long term tolerance of HLA-mismatched kidneys has recently been demonstrated in humans (Basker *et al.* 2000). Extensive research has revealed that the risk of porcine endogenous retrovirus (PERV) transmission to human patients is low (Switzer et al. 2001; 2003). RNA interference (RNAi) is a promising method for knocking down the already low level of PERV expression in porcine somatic cells. Using RNAi mediated knockdown, PERV expression has been further reduced in porcine somatic cells for 4–6 months, these cells were successfully used in SCNT and gave normal piglets (Dieckhoff *et al.* 2007a, Dieckhoff *et al.* 2008, Dieckhoff *et al.* 2007b). RNAi mediated PERV expression

knockdown provides an additional level of safety for porcine-to-human xenotransplantation. However, preliminary functional data on porcine kidneys and hearts in non-human primates is promising although the long term effect of porcine organs on human physiology is to a great extent unexplored (Ibrahim *et al.* 2006).
Biopharming in animal model is advantageous for many reasons- first it loers production cost of pharmaceuticals, second it ensures flexible supply, third the posttranslational modification is possible in animals but not in bacteria. However it is not without some key limitations such as poor scaling up and storage capacity, excessive purification concerns and a possibility of an act of biohazard (Murphy 2007).

3.2.10 Animals as model organism

Farm animals, such as pigs, sheep or cattle, may be more appropriate models in which to study the treatment of human diseases such as artherosclerosis, non-insulin-dependent diabetes, cystic fibrosis, cancer and neuro-degenerative disorders, which require longer periods of observation than is possible with mice (Hansen & Neumann 2004, Li & Engelhardt 2003, Palmarini & Fan 2001, Theuring *et al.* 1997). Because genetically modified mice do not manifest myocardial infarction or stroke as a result of atherosclerosis, new animal models, such as pigs that exhibit similar pathologies, are needed to develop effective therapeutic strategies (Rapacz *et al.* 1989). An important porcine model has been developed for the rare human eye disease retinitis pigmentosa (PR) (Petters *et al.* 1997). Patients with PR suffer from night blindness early in life due to loss of photoreceptors. Transgenic pigs with a mutated rhodopsin gene have a phenotype quite similar to the human patients and effective treatments are being developed (Mahmoud *et al.* 2003).

3.2.11Transgenic pets

Fluorescent green transgenic medaka (Oryzias latipes, rice fish) have been produced and approved for sale in Taiwan (Tanaka *et al.* 2001). The fluorescent medaka is currently marketed by the Taiwanese company Taikong. The "GloFish®"is a trademarked transgenic zebra fish (Danio rerio) expressing red fluorescent protein from a sea anemone under the transcriptional control of a muscle-specific promoter (Gong *et al.* 1991, Hall *et al.* 2007). Yorktown Technologies (www.glofish.com, July 2007) initiated commercial sales of the transgenic zebrafish in the United States with retail prices of approximately $ 5,00 each. The GloFish® thus became the first transgenic animal freely distributed throughout the USA. A recent report of the FDA contained no evidence that GloFish® represents a risk (US FDA, 2003). Commercialization of fluorescent fish has gone forward in several countries other than the USA, including Taiwan, Malaysia, and Hongkong, whereas marketing in Australia, Canada and European Union is currently prohibited.

4.0 Livestock: Bangladesh perspective (BLRI 1998; BLRI 2008; BLRI 2009; CVASU 2007; CVASU 2008)

4.1Traditional Biotechnology

Artificial Insemination: Many crosses with healthy bulls such as Indian Hariana improved local varieties immensely. Local varieties used are-

- Rajshahi – Godagari area,
- Pabna- Shahjadpur, Bera, Sadar, Santhia and Faridpur upazilas,
- Dhaka- Manikgonj, Munshigonj, Narsingdi,
- Faridpur- Madaripur, Sadar, Gopalgonj.
- Comilla- Brahmanbaria and
- Mymensingh- Bajitpur, Austagram, Kuliar char.

Hybrid Breeding: Concentrations have been focused onto the following cases-

- BLRI cattle breed-1
- Improvement of Red Chittagong cattle, Goyal and Buffalo by selective breeding
- Selective breeding of Black Bengal Goat
- Open nucleus breeding system (ONBS).

4.2Modern Biotechnology

Marker Assisted Breeding: The idea behind marker assisted selection is that there may be genes with significant effects that may be targeted specifically in selection. Some traits are controlled by single genes (e.g. hair colour) but most traits of economic importance are quantitative traits that most likely are controlled by a fairly large number of genes. However, some of these genes might have a larger effect. Such genes can be called major genes located at

QTL. Although the term QTL strictly applies to genes of any effect, in practice it refers only to major genes, as only these will be large enough to be detected and
mapped on the genome. Following the pattern of inheritance at such QTL might assist in selection.

- Improvement of Pabna cattle:
  1. Genotypic and Phenotypic characterization
  2. Genetic improvement by breeding
  3. Identifying reproductive disorder and controlling measures
  4. Cryopreservation of improved germplasm

  The result were encouraging-
  1. Coat color: red
  2. Body conformation: medium, typical horn of Pabna cattle
  3. Milk yield: 5-6 kg/d
  4. Lactation length improved
  5. Improved calves birth weight
  6. Reproductive efficiency increased

Feed improvement: several different feed alternatives have been tried such as-

- Sugarcane leaves for growing indigenous bulls: Advantageous in dry season as grasses do not grow generally, but sugarcane do. Feeing tops, pressed stock or whole cane can be used.
- Banana tree as silage or combined with other materials.
- Cottonseed cake supplementation to grow Native Bulls Fed Rice Straw
- Screening high biomass yielding grasses and form a Germplasm Bank for example: Grass + legume shows high biomass yield.
- Salt tolerant grass species: In Char areas the following salt tolerance was found-
  Napier Bajra> Splendida> Guiena> Napier arosha> Paspalum> Andropogon> Para> Ruzi> Signal> Plicatulum> Buffel> Setaria

Resistance against Disease: several vaccines have been developed locally or have been improved for local varieties. For example:

Viral:

- Foot and mouth disease vaccine
- NDV4HR for poultry disease Ranikhet
- Antiserum Antibiotic against Goat and Sheep plague/PPR (Pests des Petits Ruminants)
- Vaccine against Goat Pox (capripox)

Bacterial:

- Mastitis vaccine
- Black quarter (BQ) vaccine
- Hemorrhagic septicaemia (HS)
- Salmonella Vaccine

Parasitic infection:

- Liver fluke
- Myiasis

Molecular characterization or Diagnosis: these methods have been successful in-

- H1N1/ avian influenza virus of duck and hen by post mortem data by PCR
- Phylogenetic analysis of HPA1 virus isolates
- ELISA based technique to diagnose Foot Mouth Disease (FMD)

Cattle Fattening: Cattle fattening package is a four-steps rearing programme of male and/or infertile female emaciated cattle for harvesting their compensatory growth within a period of 60 to 120 days (Yamada *et al.* 2007). Potential transferable areas of this project are mostly of the South and South East Asian countries including Bangladesh and depending on feed price, marketing facilities, and types of animals net benefit per animal in a period of 90 days may vary from TK. 800.00 to TK. 1800.00 is estimated.

Animals with correct skeletal structure, short and squarely placed legs, short necks, broad heads, wide back and breast, loose skin and rectangular or square in shape should be collected and dewormed. Feeding formulae such as- Ad lib Urea-molasses-straw (UMS) + a concentrate mixture (a kilo dry matter of which contains 10.0 to 11.0 MJ ME and 150 to 170g CP) @ 0.8 to 1.0% of LW is used. Ad lib green grass impregnant with 5.0 to 10.0% molasses + concentrate mixture (as shown in Fi) @ 0.8 to 1.0% of LW. Ad lib UMS+ supplementary green grass + concentrate mixture (as shown in Fi) @ 0.8 to 1.0% LW. Ad lib straw (preserved in fresh and wet condition) with 2% urea impregnant with 5.0 to 10% molasses + concentrate mixture (as shown in Fi) @ 0.8 to 1.0% LW. Collection and

preservation of feeds during production seasons, using locally available feed ingredients for formulation of diets and feeding a higher quality diet initially and minimization of feed cost at the later stage of rearing are the factors for formulation of cost effective diets. Market demands on the size and live weight of animals, fatty, age and colour are the few factors for consideration to achieve a good market.

Banana Foliage Processing and Preservation for Feeding Cattle: Banana plant is grown throughout the country, especially in Barishal, Patuakhali, Khulna, Jessore, Tangail, Mymensingh, Rajshahi, Dinajpur, Rongpur, Rangamati, Khagrachari, Bandarban and Chittagong district. About 3418 ha of land is under Banana cultivation. A hectare of land yields about 69.3 MT of biomass annually. of which, 17.3 MT is fruit and the rest 52 MT/ha is banana pseudostem and leaves (banana foliage). After harvesting Banana fruits Banana foliage may be used as feeds for cattle. Banana foliage is available in large amounts in the wet season. It may be processed and preserved for effective utilization as cattle feed immediately after the fruit harvest and may help mitigation of cattle feed shortage, especially, in that particular banana growing areas.

Utilization of banana foliage as cattle feed helps to alleviate pollution of the environment due to its aerobic fermentation in the traditional system.

Algae as an Alternative Feed for Ruminants on Straw - based Diet: Microscopic green algae can be grown in shallow sink with very little cost and land. This can be fed to animal, which can supply protein, vitamin and minerals. In most tropical and sub tropical areas including Bangladesh this use has huge prospect in the coming years. Production of algae cost only Tk. 0.05/L of suspension. Due to rich source of protein, vitamin and minerals, algae can be fed as a substitute of protein meal and grasses in ruminants.

Algae are autotrophic organisms, the size of which can vary from unicellular to giant oceanic species. Chlorella, Scenedesemus, Padyastrum, etc. are the unicellular algae rich in protein, vitamins and minerals. They can be grown very quickly in a shallow fresh water sink, which could be used for feeding livestock. The culturing techniques are as.

Livestock Feed and Fuel Production from Cultivation of Ipil Ipil: Livestock feed containing 23% to 24% crude protein and fuel could be produced from a single piece of land. Feed and fuel could be harvested for at least three years after cultivation and require minimum production costs. All plain and cultivable land except that of flood prone areas slopes of roads and barrages and hillocks (except acid soils) can be used for this cultivation.

Maize and Cowpea Mixed Forage Production and Utilization: Harvesting more nutritious forage biomass from a single area of land help maintaining soil health and the environment. All types of loamy soils can be used as a potential area for this technique. The benefit/cost involvement was not calculated directly but Table 4 shows feeding responses of maize single or mixed biomass to daily milk yield of cows.

Table 3: Responses of different biomass feeding to daily average milk yield

Diets	***Daily average milk yield (Litre)***
Maize + Cowpea	*3.11*
Maize+Cowpea+2.50 Kilo/head concentrate mix	*4.62*
Dry straw + 5.0 Kilo/head concentrate mix	*4.48*

Table 3 shows that feeding only maize & cowpea mixed may support daily 3.11 litres of milk yield of a Pabna cow. Supplementation of the mixed forage with daily 2.5 kilo concentrate mixture supported daily average milk yield of 4.62 litres and it was higher than the daily milk yield found from feeding dry straw and 5.0 kilo concentrate daily.

Cultivation of maize and cowpea yields more biomass with higher nutritive value and helps to maintain soil health as compared to only maize fodder cultivation.

Production of maize and cowpea from a single piece of land reduces fertilizer dosages and helps to maintain soil health. Feeding mixed forage biomass supported better nutrition to animals & higher daily milk yield. The technology is friendly to the environment.

PRR Vaccine: PPR homologous vaccines are naturally avirulent but highly immunogenic and produce solid immunity. Use of this live homologous vaccine will save the life of 32 millions goat throughout the country. Hence will boast up the national economy through the establishment of more goat farms in this country. The cost of each dose of vaccine will not more than Tk. 2.00 but will save a goat of values more Tk. 1000.00. So far no alternate technique has developed yet to compare with this one.

By inactivating virulent virus in the host system, this technique will reduces the transmission of virulent virus in the environment, hence friendly to the environment.

Mycoplasma Antigen: Avian mycoplasmosis is an emerging disease of Poultry in Bangladesh. Low egg production in layer, poor hatchability and least feed conversion ratio in broiler leading the farm uneconomic due to this disease and thus hindering the commercial poultry operation. The disease is very difficult to diagnosis clinically, always confused with other respiratory disease but serologically it can be detected easily using this developed antigen.. The assay is a very cheap and cost-effective one. This antigen can be used as routine diagnosis of Mycoplasma infection, hence it will be helpful to control the disease and to improve the socio-economic condition of the farmers as well as for the country.

ELISA - Based Technologies for the Diagnosis of FMD Virus and Vaccine Evaluation: ELISA-based technique is highly sensitive, accurate, reliable and precise. 24 samples can be tested in each plate within 3 hours. This technique is prerequisite for the control of FMD though the quick diagnosis of field virus. This technique can safe both time and cattle population from FMD and is highly economic compared to other technique like complement fixation, neutralization and animal inoculation tests.

16s rRNA based PCR, DGGE and TGGE to characterize rumen bacteria: Rumen bacterias vary from zone to zone. Local rumen bacteria will vary significantly from the foreign ones. Characterizing local rumen bacteria can be useful to design good vector for producing probiotics or to degrade antinutrient or allergen in cattle rumen and thereby conferring proper digestion.

Vaccine against major zoonotic disease through meat: *Clostridium jejuni/coli, L.monocytogens, Salmonella, Campylobacter, Yersinia enterocolitica* are some of the bacterias that are transmitted through meat. They are called Zoonotic pathogens. This pathogens can be identified by biosensors.

Poultry Hybrid: The poultry population is increasing at the rate of 6.5%per year and there are three distinct systems of poultry rearing: scavenging, semi-scavenging and intensive. The scavenging system is characterized by poor nutrition (only 14% and 23% of the requirement for protein and energy, respectively), lower productivity (35-45 eggs/year) and high (45-84%) mortality, but yet it contributes over 85% of poultry products in Bangladesh. Semi-scavenging is a model of poultry rearing developed in this country. Intensive poultry farming techniques have not yet been fully adopted. Thus efforts have been made for developing a breed or strain suitable for scavenging and semi-scavenging systems of rearing. Of the six distinct types of pure native chicken studied, native Naked Neck, native Dwarf and Hilly were found to have better production potentialities than others. Among different combinations of native and exotic chickens, RIR x Fayoumi, NN x RIR and NN x Fayoumi had better performances under semi-scavenging conditions. In all cases productivity under scavenging conditions was lower than under intensive systems largely due to poor nutrition. Supplemental feeds, especially protein sources increased the productivity of scavenging and semi-scavenging chicken. The paper concludes with a summary of research requirements.

Cattle breeding: On the basis of various studies made during 1973-1976 a Breeding Policy was devised in 1976 which was approved by the government in 1982. The policy is to:

(a) Breed females of the urban, semi-urban and milk pocket areas with ½ Friesian – ½ Sahiwal bulls,

(b) Breed females of the rural areas with ½ Friesian – ½ local bulls.

However, frozen H. Friesian semen in the district level and milk pocket is also being used.

Hilsha in pond: Hilsha is a local variety of fish whose natural habitat is salt water however, it comes to lay its eggs on rivers. Researchers showed Hilsha with a modified feed (TSP and cow dung) can grow in ponds.

5.0 Conclusion

Genetic engineering in animal model has attained much hype, yet not without some serious bioethical complications. Are we blurring the lines between species by creating transgenic combinations and are these genetic modifications is resulting in any grave health concerns are the questions often asked by critics. Although it is true that there is a certain bit of risk involved with the technology, this is an utmost reality that to feed a the ever growing population of the world accepting this technology is an absolute compulsory; there is no going back from here. Especially for countries like Bangladesh where scarcity of forage lands are making things extremely harsh for livestock raising, the technology is definitely is the silver lining; offering a possibility that even a few decade ago was unimaginable.

Nevertheless, the progress Bangladesh has achieved in this field has not been of much to talk about. Apart from some improvement in developing local formula for cattle fattening feed, there has neither been much done nor been much initiated as far as genetic engineering is concerned. However, the potential are immense. With an emerging pharmaceutical industry, it is matter of time before the industry finally takes up genetic manipulation as a way to go forward. And with that it does not seem improbable that handsome investment would also be put forward in the fields of biopharming and others.

Salinity has also been a problem in the southern part of the country. So designing fishes that can withstand the saline water and still be fertile would be an interesting prospect for Bangladesh to deal with. Improving meat by classical breading has long been the only avail for livestock farmers. However, more government incentives must be placed to initiate researches on genetic manipulation and spread the transgenic improved cattle country-wide. However, it is to be mentioned that without an integrated effort from both the government and private sector any significant improvement in recombinant DNA technology can hardly be achieved.

In overall analysis, Bangladesh does hold an unexplored field of genetic manipulation in animal model. Hand down, more emphasis must be put to launch a massive campaign to popularize biotechnology among livestock farmers and undertake necessary steps to assist them accordingly.

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