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Review Paper

Recombinant DNA technology of hormones and vaccines in maximizing livestock production and productivity: A review

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Abstract. Recombinant DNA technology of hormones and vaccines is in favour of generating multiple purposes in maximizing animal production and productivity. For instance, the Booroola Fecundity gene (FecB), which is found in a region of ovine chromosome 6 increases ovulation rate by 1.5 to 1.6 and one to two extra lambs. The gene responsible for the double muscling trait which is found in bovine chromosome 2 enhances muscling rates. The genetically engineered hormones such as human (HST), bovine (BST) and procine (PST) somatotropin, interferon, and lymphokines are on stage to be marketed for use in human and veterinary medicine. DNA Viruses are genetically engineered to produce vaccines against diseases. Gene-deleted vaccines such as salmonella vaccine for sheep and poultry, a pseudorabies virus vaccine for pigs, salmonella enterica serovar typhimurium and serovar enteritidis vaccines in poultry and streptococcus equi vaccine in horses, bovine herpesvirus-1 marker vaccine in cattle are used in disease prevention. The main challenges facing the recombinant DNA technology of hormones and vaccines are the cost of production, human health concern of genetically engineered animals as well as violating species barriers or “playing God”.

Keywords: DNA vaccines, hormones, productivity, recombinant DNA.

INTRODUCTION

Recombinant DNA technology, also known as genetic engineering is a means of altering the genes in a living organism to produce a Genetically Modified Organism (GMO) with a new genotype. It is a technique used for direct genetic modification of organisms or population of organisms using recombination of DNA. The techniques are used to identify, replicate, modify and transfer the genetic material of cells, tissues or complete organisms (Karp, 2002). This technique involves the capacity to isolate, cut and transfer specific DNA pieces, corresponding to specific genes (Klug and Cummings, 2002). Besides increasing basic knowledge about mammalian genetics and physiology, including complex traits controlled by many genes such as many human and animal diseases (Eggen, 2003), researches in genetic engineering of animals are oriented towards a variety of medical, pharmaceutical and agricultural applications.

The recombinant DNA (rDNA) technology in animals can be used to produce pharmaceuticals in eggs (Harvey et al., 2002) or milk expressing novel genes in the mammary glands of livestock (Wright et al., 1991), or fibers such as spider silk in milk. Genetically engineered poultry, swine, goats, cattle, and other livestock are beginning to be used as generators of pharmaceutical and other products, potential sources for replacement organs for humans, and models for human disease besides being used as the source of humans food (Maga and Murray, 1995). Milk, egg white, blood, urine, seminal plasma, and silkworm cocoons from transgenic animals are candidates to produce recombinant proteins, enzymes and clotting factors on an industrial scale

(Houdebine, 2002).

The genetically engineered hormones such as human Somatotropin (HST), bovine Somatotropin (BST) and porcine Somatotropin (PST), interferon, and lymphokines are on stage to be marketed for use in human and veterinary medicine. The hormones enable to correct growth retardation and make to grow faster with less fat and leaner production of dairy cattle (Sudharsan, 2006). Growth hormones (GH) are economically important in genetic improvement, artificial insemination, embryo transfer and others. On the contrary, many hormones from recombinant bovine somatotropin in milk, porcine somatotropin in meat and steroid hormones can be retained in or drained in to ground water through animal excreta. These cause ground water pollution as well as cancer, reproductive effects and endocrine disruption to human being (Kolodziej et al., 2004).

As genetically engineered hormones are in favour of generating multiple purposes in maximizing animal production and productivity, recombinant DNA technology is also expected to revolutionize vaccine development in the future for maximising production and productivity of animals. DNA vaccines contain those genes of the pathogen which produce the antigen are expected to combine the effectiveness of live vaccines with the comparative safety of those based on killed pathogens. One of the greatest advantages is the ability of DNA vaccines to induce both humoral and cell-mediated immune responses, which is critical for protection from many diseases. DNA vaccines can induce long-term immunity and be repeatedly administered without the interference of antibodies. The safety of DNA vaccines has been established in various trials in several species including humans (Kim et al., 2001). However, recent progress has resulted in the development of DNA vaccines in a number of out bred target species (Carvalho et al., 2009; Redding and Weiner, 2009). The use of a needle-free vaccine delivery device was shown to reduce the effective dose of an experimental polyvalent DNA vaccine for avian influenza, and to rapidly deliver repeated injections in poultry (Rao et al., 2009).

A number of commercially available vaccines are based on DNA virus vectors. Of these poxviruses and herpes viruses have been successfully licensed for use in veterinary medicine (Gerdts et al., 2006). Fowl pox and canary pox vectors have been used in a wide range of applications (Swayne, 2008) whereas; replication defi-cient human adenovirus vectors have been used very successfully in the development of foot and mouth disease vaccines (Rodriguez and Grubman, 2009).

Therefore, the objective of this review is to quantify the role of genetically engineered hormones and vaccines / recombinant DNA technology/ in maximizing livestock production and productivity with the following specific objectives:

1. To quantify the roles of recombinant DNA technology of hormones in maximizing animal production and productivity.
2. To quantify the potential applications of recombinant DNA technology of vaccines in maximizing animal production and productivity.
3. To identify the challenges of using recombinant DNA technology hormones and vaccines.

Recombinant DNA technology

The assembly of recombinant DNA by restriction enzyme cutting and re-ligation was a crowning achievement of biology in the 20th century (Backman, & Ptashne, 1978). Homologous recombination is intricately involved in DNA replication and repair of eukaryotic and prokaryotic organisms (Yu et al., 2003). Recent developments have provided new insights for the use of homologous recombination in gene therapy and in vivo genetic engineering (Court et al., 2002). DNA vaccines (DNA clone) usually 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). The recombinant plasmids containing a foreign gene are purified from the bacteria, and the “naked” DNA is injected directly into the animal, usually intramuscularly or intradermally (into the skin). The animal’s cells take up the DNA, and an immune response is induced to the protein expressed from the foreign gene. Several types of vectors are available for cloning large fragments of DNA. Library clones are screened to identify the particular clone that carries the DNA of interest (Hayes, 2003). Small plasmids have been identified which consist of a replicon and very little extraneous DNA sequences (Hayes, 2003).

Roles of recombinant DNA technology in increasing fertility and hypertrophy

Booroola gene

The Boorola fecundity gene (*FecB*) is a single autosomal gene, which increases ovulation rate and litter size in sheep. The *FecB* locus is situated in the region of ovine chromosome 6, which is synonymous to human chromosome 4 (Montgomery et al., 1996; Ghaffari, 2009). The effect of *FecB* mutation is additive for ovulation rate and each copy of *FecB* increases ovulation rate by 1.5 to 1.6 and one to two extra lambs (Piper and Bindon, 1996; Davis, 2004).

Double muscling (hypertrophy)

The genomic mapping approach has identified many

Quantitative Tri Loci (QTLs) for production traits in livestock. Understanding the molecular basis of double-muscling has opened up the possibility to select either for or against this phenotype directly at the genotypic level.The gene responsible for the double muscling trait is identified in a region on bovine chromosome 2 as the most likely location of the double muscling gene. The calving difficulties associated with double- muscling have led several breeding organizations to treat this condition as a genetic defect that needs to be eliminated. In specific economic contexts, however, the gains in feed conversion ratio, dressing out percentage and meat quality (increased lean and tenderness) have outweighed the costs of dystocia, leading either to a systematic selection for double-muscled animals or their use in cross breeding (Hanset, 1991).

Genetic engineering of hormones for maximizing production and productivity

Subsequent efforts to genetically alter growth rates and patterns have included production of transgenic swine and cattle expressing a foreign c-ski oncogene, which targets skeletal muscle, and studies of growth in lines of mice and sheep that separately express transgenes encoding growth hormone-releasing factor (GRF) or insulin-like growth factor I (IGF-I) (Murray et al., 1999).

The hormones of an animal origin are drugs from placenta of cows, cow's colostrums and colostrums drugs (Habbibulin, 1998) which contain natural hormones such as progesterone, estrogen, gonadotropin, prostaglandins, and also vitamins, trace substances and other biologically active materials. It promotes fast regeneration of the broken functions by diseases of the reproductive organs and reduces twice the dose of expensive synthetic drugs by their joint use (Shubina et al., 1996). Growth hormones play crucial role in reproductive technologies on the genetic improvement of livestock such as artificial insemination, embryo transfer and others used to disseminate superior gene of desired trait where the economic returns are significant (Mada, 2005).

Growth Hormones act by interacting with a specific receptor on the surface of cells. For example: increase in height of animals is the most widely known effect of GH is stimulated by at least two mechanisms. Initially it is because of fat-insolubility of polypeptide hormones that they cannot penetrate sarcolemma. Thus, GH exerts some of its effects by binding to receptors on target cells, where it activates the MAPK/ERK pathway (Binder et al., 2007).

Oestrus synchronization and conception rate improvement with different stimulants and use of Gonadotropin-releasing hormones (GnRH) followed seven days later by prostaglandin F2α (PGFalpha) (Schmitt et al.,1996). The use of recombinant bovine somatotropin (rBST) in dairy cows increases both milk yield and production efficiency and decreases animal fat (Madan, 2002). A porcine somatotropin hormone has been developed that increases muscle growth and reduces body-fat deposition, resulting in pigs that are leaner and of higher market value (Adel et al., 2007).

Genetically engineered DNA vaccines in maximizing production and productivity

Immunization through DNA vaccines is an alternative immune response (Ramsay et al., 1999). For example, DNA Viruses can be genetically engineered to produce vaccines against diseases such as dental caries; and life-threatening infections like diarrhea, AIDS, etc. (Moffat, 1995). Vaccination may have both favourable and unfavourable consequences. Live vaccines for example can revert back to pathogenic organisms and produce disease or, even death (CAST, 2008). However, the development of rDNA technologies has provided new ways of attenuating disease agents by modifying or deleting their genetic makeup, or genomes, to create safer, more efficacious vaccines. With the development of new, more efficacious, stable, and safe recombinant vaccines, delivery methods and immuno-stimulating adjuvant compounds that enhance the immune response have been also studied (CAST, 2008).

Gene-deleted vaccines

The availability of recombinant DNA technology has facilitated the creation of specific gene-deleted pathogens for use as live vaccines. In live genetically modified vaccines, 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 or gene-inactivated vaccines are developed to attenuate the disease agent. In these conditions, 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). Examples of such gene-deleted vaccines include a *salmonella* vaccine for sheep and poultry and a pseudorabies virus vaccine for pigs. Gene-deleted *salmonella enterica* serovar Typhimurium and serovar *Enteritidis* vaccines have been licensed for use in poultry (Babu et al., 2004; Meeusun et al., 2007) and similarly, gene-deleted *streptococcus equi* vaccine has been licensed for use in horses (Jakobs et al., 2000; Meeusun et al., 2007). A double gene deleted pseudorabies virus marker vaccine has been licensed for use in pigs (Ferrari et al., 2000; Meesun et al., 2007) and deleted a bovine herpesvirus-1 marker vaccine has been licensed for use in cattle (Meesun et al., 2007; Van Oirschot et al., 1996). Foreign genes from disease agents have been inserted into potatoes, soyabeans, and corn plants and fed to

animals; the expressed proteins from those foreign genes immunized the animals against the disease agent (Streatfield, 2005).

Subunit vaccines

Recombinant inactivated vaccines are subunit vaccines which are commercially available for respiratory pathogens such as *Mannheimia haemolytica* and *Actinobacillus pleuropneumoniae* based upon the leukotoxins produced by these organisms, as well as transferrin-binding proteins. *Actinobacillus pleuropneumoniae* is an excellent example of a vaccine composed of subunits selected on cross-serotype reactivity, thus providing broad-spectrum protection against disease. Likewise a vaccine against atrophic rhinitis containing a non-toxic derivative of *Pasteurella multocida* dermonecrotic toxin produced by a genetically modified *Escherichia coli* strain together with a conventional *B. bronchiseptica* bacterin Vaccines against CSF demonstrate well the need to target recombinant technology to a particular purpose. Because subunit vaccines do not replicate in the host, they usually are administered (injected) with an adjuvant, a substance that stimulates the immune system of the animal to respond to the vaccine. Adjuvants can enhance the response to a vaccine by protecting the vaccine from rapid degradation in the animal (CAST, 2008).

Genetic vaccines (vaccines produced from chimeric viruses)

Chimeric pestiviruses have been developed using an infectious complementary DNA (cDNA) clone containing the classical swine fever virus (CSFV) genome. The virus was constructed by replacing the CSFV E2 coding sequence in the infectious DNA copy of CSFV vaccine strain C with the corresponding E2 coding sequence from BVDV (van Gennip et al., 2000). These chimeric viruses appeared to be attenuated in pigs, induced complete protection against CSFV challenge and helped to discriminate between vaccinated and infected pigs (Reimann et al., 2004; van Gennip et al., 2000).

Challenges in using recombinant DNA hormones

Effects on the animal health

The use of Recombinant Bovine Growth Hormone (rBGH) had problems like mastitis, lameness, loss of condition, and lowered immune system functions, which they attributed to rbST use (An and Butler, 2008). Reduced pregnancy rates, increase in days open, increased incidence of retained placenta, decreased gestation length and birth weight of calves are also some of the problems observed in using rBGH. The use of synthetic hormones has numerous negative consequences: interruption of natural hormone status, change in quality of products due to accumulation of hormonal drugs in meat and milk of animals (Kistanova, 2003). High-yield milking cows due to BST show a greater incidence of mastitis than lower-producing cows (Judge et al., 1997) and having fewer calves per lifetime.

Cattle with an oestrogen implant are adversely affected by hot climatic conditions and managing heat load in feedlot cattle is crucial to animals' welfare (Gaughan et al., 2005). In beef cattle production the use of hormone growth promotants can causes uncommon occurrence of chronic stress condition as signs of poor welfare (Marin et al., 2008). This is occurred when hormonal implants interact with the animal's natural hormones.

Effects on the environment

Livestock farming is thought to be the major source of steroid hormones found in regional groundwater (Peterson et al., 2000), number of streams and rivers and external surface water (Kolpin et al., 2002). Beef cattle wastes are strongly androgenic (Durhan et al., 2006). Significant amounts of synthetic and natural hormones and their metabolites are excreted in animal waste (Kolok and Sellin, 2008). Synthetic hormones excreted by animals are present in manure applied as fertilizer and in feedlot retention ponds, and from there they may be retained in soil or transported to ground and surface water (Khan et al., 2008a, b). Lange et al. (2002) calculated the number of beef cattle implanted with estrogens and androgens or progesterone, and the percent of applied hormone that reach the environment via cattle excrement. Commonly used androgenic growth promoter trenbolone has been found in groundwater near cattle feedlots, and that this growth promotor has androgenic effects. These numbers represent an increase in estrogens and androgens or progesterone over natural elimination rates (Lange et al., 2002).

Effects on human

Humans are potentially exposed to the synthetic hormones by consumption of commercial meat products and from environmental exposures related to animal waste (NRP, 2006). Human exposure to both the synthetic and natural hormones causes cancer, reproductive effects, and other endocrine disruption outcomes. Estrogen is carcinogenic, anabolic steroids are reproductive toxicants and trenbolon is anabolic steroid. TBA, zeranol, and MGA cross the placenta and are detectable in fetal tissues in rabbits (Lange et al., 2002a) and reflected in humans. Some evidences showed that xenobiotic growth promoters and their metabolites are thought to be genotoxic (Metzler and

Pfeiffer, 2001). Veterinary use of hormones causes postmenopausal women, and pre-pubertal children, leaving them more vulnerable to the effects of exogenous hormone exposure (UK VPC, 2006).

Challenges in using recombinant DNA vaccines

In the recombinant DNA technology, the genes for the desired antigens must be located, cloned, and expressed efficiently in the new vector, but the cost of production is high. When engineered vaccine virus is used to vaccinate, care must be taken to spare immuno-deficient individuals. On the other hand, the potential integration of DNA into host genome leads to insertional mutagenesis. Induction of autoimmune responses: anti-DNA antibodies may be produced against introduced DNA. Induction of immunologic tolerance: The expression of the antigen in the host may lead to specific non-responsiveness to that antigen. Antibodies produced against the subunit may not recognize the same protein on the pathogen surface. Isolated protein does not stimulate the immune system as well as a whole organism vaccine.

Genetic engineering of animals is violating species barriers or “playing God” and interferes with the integrity or telos of the animal (Holland and Johnson, 1998). The “double-muscle” trait reduced width of the cow’s pelvic passageway (Van denheede et al., 2001).

The super pig, a product of genetic engineering, is a sick animal, fattened artificially by human growth hormone. This super pig must endure side effects including crippling arthritis and distorted vision caused by the human growth genes that makes them cross-eyed, so on, in addition to factory pig farms, there will be pig organ farms (Chad West, 2006).

CONCLUSION

From the review, it was possible to see that recombinant DNA technology in animals can meet the growing global demand for high quality and safe animal food products in a sustainable, environmentally safe and positive animal welfare manner. Specifically, it was possible to deduce that recombinant DNA technology of hormones and vaccines can enhance production and productivity of farm animals. Genetically engineered hormones played crucial role in reproductive technologies of genetic improvement of livestock. Artificial insemination, embryo transfer and others used to disseminate superior gene of desired trait where the economic returns are significant. The development of recombinant DNA technologies has also provided new ways of attenuating disease agents by modifying or deleting their genetic makeup, or genomes, to create safer, more efficacious vaccines. Due to the outlook for significant benefits there is ample global research and private development of genetically engineered animals that improve foods, are environmentally friendly, improve animal welfare and produce industrial products. Genetic engineering may improve several aspects of livestock production including milk quality, meat production as growth and carcass composition, animal welfare (via disease resistance), reproductive performance and quality of hair and fibre.

RECOMMENDATIONS

So as to use recombinant DNA technology of hormones and vaccines in increasing livestock production and productivity:

1. Stress created on genetically engineered animals should be minimized.
2. Concerns raised on public health and environmental issues should be given attentions.
3. The lead of the science, rigorous regulatory approval should portend compelling consumer benefits and sustainable production.

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