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Popular Article

Genetically Modified Meat: Transgenesis and Cloning

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Abstract

Meat can be defined as "the muscle tissue of slaughter animals". It is one of the most significant human food sources since it contains a high amount of useful protein as well as important micronutrients that are necessary for human health. The FAO estimates that meat consumption in developed countries might reach 100 kg per person per year by 2030. Meat demand is expected to expand at a rate of 1.3 percent each year until 2050. According to the International Food Policy Research Institute, annual per capita meat consumption in high-income countries is expected to rise from 90 kg per person per year to over 100 kg per person per year between 2000 and 2050, while low-income countries' consumption is expected to rise from around 25 kg per person per year to nearly 45 kg per year. The majority of the growth is projected to occur in countries with a middle or low income. However, there is a maximum to conventional meat production beyond which there would be high demand without any sources. As a result, prices will rise and global food distribution will deteriorate. The conventional meat industry, on the other hand, has the ability to adopt and harness accelerated genetic selection, cloning, and genetic modification technologies in order to not only increase production capacity but also to better meet consumer demands for quality animal welfare, sustainability, and healthiness. It would also provide the sector with additional flexibility and ability to improve the quality of the product given to consumers while also increasing efficiency and output.

Concepts of Genetic research

Biotechnology, as employed in a transgenic event, is known as transgenic cloning. Food demand is rising due to population growth; as a result, the production of inexpensive but nutritious foods for a large population, as well as the need to boost farm animal sustainability, may inspire food production from genetically modified animals in the future. Cloning, transgenesis, and transgenesis

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followed by cloning are examples of biotechnology in animals that have the potential to increase the quality, yield, and safety of food items by direct genetic manipulation. Transgenesis is the act of altering an animal's genome by introducing (through gene transfer) a new or foreign gene (i.e., DNA) not found in the recipient species, or deleting or modifying an endogenous gene with the purpose of producing an animal with a beneficial function or superior trait (e.g., adding a gene that promotes muscle growth). The transgene is the gene or genes that are transmitted or changed (TG). Cloning is a technique for creating genetically identical duplicates of a specific species (i.e., one which possess high breeding value). The process of generating a clone whose donor cells include heritable DNA inserted via a molecular (Key et al., 2008).

Protocol of transgenesis

The process of transgenesis involves series of steps namely,

- Selection of the genetic trait to be transmitted, which is the first stage in the transgenesis process. In action, the character's gene must be additive or dominant.
- Development of a gene transfer vector.
- Gene transfer to the target cell
- DNA analysis is used to identify transgenic organisms. Crossing homozygous transgenic to hemizygous and transgenic to non-transgenic mice to test gene expression stability.

Techniques in transgenesis

The introduction of exogenous genes into recipient embryo tissues in order to enable their transfer into the germ line has resulted in the creation of several approaches. Among the approaches used are

Use of vector

- **DNA microinjection**
- **Use of chimeric method**
- **Laser method**

Trans-genesis in the improvement of production traits

Trans-genesis technology has the potential to modify economically important traits in a quick and accurate manner. In contrast to 'traditional' selection programs, knowledge of the genes that govern these traits and their regulation is required.

Growth and meat traits

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The metallothionein promoter was used to control the expression of growth hormone in most previous studies in domestic animals (pig, sheep, and rabbits). Production of transgenic swine and cattle expressing a foreign c-ski oncogene, which targets skeletal muscle, and studies of growth in mouse and sheep lines that each express transgenes encoding growth hormone-releasing factor (GRF) or insulin-like growth factor I were among the subsequent efforts to genetically alter growth rates and patterns (IGF-I). Inducible promoters, such as the metallothionein promoter, which may be triggered by zinc supplied in the animal feed, have been utilized to offset the deleterious effect of growth hormone synthesis.

A gene producing myostatin, a growth factor that generally inhibits the growth of skeletal muscle cells, has been found in several cattle breeds. So, by using genetic alteration to limit myostatin function, we can see an increase in muscle development and differentiation.

A diet high in omega 3 fatty acids has been hailed as a cure for cancer, autoimmune diseases like arthritis, among other ailments. Omega 3 fatty acids are poly unsaturated fatty acids with a long chain found primarily in fish. These fatty acids are not produced by humans or farmed animals. Although agricultural animals' tissues contain significant levels of omega 6 fatty acids, they lack the enzymes (n-3 fatty acid de-saturase) needed to convert them to omega 3 fatty acids. By inserting a double bond into the hydrocarbon gene, the fat-1 gene desaturase found in the roundworm C. elegans3 converts omega 6 fatty acids to omega 3 fatty acids. So, using the nuclear transfer approach, fat-1 gene desaturase was used to create fat-1 transgenic pigs. In comparison to non-transgenic pigs, transgenic pigs had a 3 times higher amount of n-3 fatty acids and a 23% lower level of n-6 fatty acids.

Although transgenic pigs and lambs with high amounts of serum growth hormone were produced, no increase in their rate of growth was seen, and only in some lines did average daily gain increase with the addition of high-protein diets. The best results were seen in the area of body fat reduction. These animals were found to have a variety of significant diseases as well as a dramatic loss in reproductive potential. Frequently, the promoters used did not allow for effective transgenic expression control. It was determined that more complicated constructs are needed to more accurately activate or repress the transgene's expression, and conflicting results were discovered about the influence of a growth hormone construct in sheep on growth and meat quality (Clark 2003).

Alteration of the lipid composition of meat by transgenesis and cloning

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Pigs are used in the majority of studies involving the use of genetic engineering approaches to alter the lipid content of meat. A spectacular case of transgenesis in which a plant transgene was introduced into the genome of a mammal is one of the earliest of this type of experiment. Saeki et al. (2004) created transgenic pigs that produced the 412 fatty acid desaturase gene (fad-2) from spinach by microinjecting foreign (plant) DNA into the pronuclei of zygotes (Spinacia oleracea). LA (C18:2 n-6) and ALA (C18:3 n-3) are required for appropriate growth and development, and this enzyme is required for their production. LA and ALA are not generated in mammals and must be obtained through nutrition, but they can be created in plants by the enzyme A12 fatty acid desaturase, which can catalyze the conversion of oleic acid to LA or ALA. The fatty acid content of lipids in modified pig tissues was considerably changed by transgenesis, with white adipose tissue of transgenic pigs containing 20% more LA than wild pigs.

Because the authors generated four transgenic pigs of the third generation, all of which were bred by a transgenic female and grew to be fertile, their findings suggested that the desaturase transgene may be passed down to future generations. It should be noted, however, that functional expression of a desaturase gene was only obtained in white adipose tissue in this work, which is fairly irrelevant to the idea of consuming transgenic meat, and that the transgenic founder female pig became agalactic and was unable to raise piglets.

A early projects (Lai et al, 2006) aimed to produce a generation of cloned pigs that expressed a humanized roundworm C. elegans fat-1 gene, which produces n-3 fatty acid desaturase. Electro-poration was utilized to transfect the fat-1 transgene into early passage male primary foetal fibroblast cells, which were then used to clone fat-1 transgenic pigs by nuclear transfer (Lai et al. 2006). Six of the ten live piglets obtained this way tested positive for the fat-1 transgene. It should be noted, however, that three of the transgenic piglets experienced symptoms of heart failure, which the authors believe was more likely due to the cloning procedure (incomplete nuclear reprogramming) than the fat-1 transgene's effect. Desaturase expression was validated in transgenic piglets' body lipids, resulting in a significant rise in n-3 PUFA concentration.

As a result, the total n-3 PUFAs (ALA, EPA, DPA, and DHA) content in skeletal muscle from transgenic pigs was quite high (about 8%), much higher than in regular pork (around 1%). Because the n-6 PUFAs concentration in transgenic pigs was lower, the total PUFAs content in these

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animals did not increase, which is a significant point because increasing total PUFAs is a factor that negatively affects pork quality.

The successful cloning of a pig with the fat-1 gene was the next step in the research with transgenic pigs. This method yielded ten clones with considerably lower n-6/n-3 PUFA ratios in meat lipids when compared to control animals (Li et al, 2006).

Pan et al. (2010) used somatic cell nuclear transfer to construct transgenic pigs with a synthesized fatty-acid desaturase-1 gene (sFat-1) from C. briggsae, a different roundworm species. Ren et al. (2011) wanted to see if the fat-1 gene from C. briggsae could be expressed in transgenic pigs and if so, how well. Pronucleus microinjection was used to inject a gene construct into embryos, and the resulting first-generation transgenic pigs were mated with wild pigs to produce the next generation.

The lipid profile of first-generation animals was considerably altered by transgenesis, with the n-5/n-3 ratio dropping from 14.53 in normal pigs to 2.62 in fat-1 transgenic pigs (Ren et al., 2011). Zhou et al. developed a generation of cloned pigs expressing the fat-1 gene from C. briggsae, which encodes n-3 fatty acid desaturase (2014). The functional expression of the desaturase transgene was shown by lipid profiles, and first-generation transgenic pigs produced high quantities of n-3 PUFAs, resulting in a much lower n-6/n-3 PUFA ratio.

Richards et al. (2011) set out to determine the fatty acid composition and oxidation indices in meat from transgenic fat-1 pigs. In comparison to the control group, fat-1 animals' loin meat was a rich source of beneficial n-3 LCPUFAs (EPA, DPA, DHA) and had roughly five times the amount of these acids. During storage, there was no evident influence of fat-1 technology on lipid oxidation stability (values of lipid peroxide and thiobarbituric acid reactive chemicals).

Transgenes that could improve the lipid content of cattle meat are being looked for not just in the genomes of other animal species, but also in the genomes of fungi and plants. The sdd17 gene, for example, was recently identified from cells of the fungus Saprolegnia diclina and has been linked to the possibility of its use in animal transgenesis.

Transgenesis, whose major goal is to improve animal development performance, can also have a considerable impact on the composition of lipids in muscle tissue in cattle. This includes, for example, transgenic pigs with the bovine growth hormone (somatotropin) transgene added to their genomes. Aside from the basic effect of this transgene in the organism, which is an increase in BW

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gain and feed conversion ratio in somatotropin-overexpressed pigs, the carcasses of transgenic pigs had a significantly lower fat content (by 64 to 85 percent) than control animals (Pursel and Solomon, 1993).

Furthermore, transgenic animals' carcasses contained 85 percent fewer saturated fatty acids (SFAs), 91 percent fewer monounsaturated fatty acids (MUFAs), and 66 percent fewer polyunsaturated fatty acids (PUFAs) (PUFAs). It should be noted that the SFA: MUFA: PUFA ratio in transgenic pig lipids was 1:1:1, which is comparable to the National Research Council's (NRC) recommended ideal values.

Cloned meat comparison

More than 1000 regularly used indicators, including as amino acid composition, fatty acid composition, meat-to-fat ratio, organ weight, and organ histopathology, all fell within the food industry's normal norms and were not significantly different from meat from naturally generated cattle. The genetic variations between cloned and natural animals are most noticeable during the embryonic phases of development, according to cloned meat. When a cow reaches maturity, for example, the variances are so minor that they have little or no effect on the meat quality.

Table: 1 The relative abilities of artificial meat to meet the demands of the market place

Market acceptability		Modified meat (genetically modified and cloned organisms)
Sustainability	Resources used	Reduced, depending on the product
	Waste	High
	Greenhouse gas emissions	Reduced
Health		Improved fatty acid profile, improved vitamin and mineral content
Safety		Reduction or elimination of zoonotic disease
Market acceptability	Capacity for mass production	Moderate technological barriers at present
	Need for further research	Moderate
	Cost	Expensive premium product
	Government Regulation	Severe restrictive regulation
Addresses welfare concerns		Moderate
Acceptability to consumers		Technophobia

(Source: Sarah et al., 2015)

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Advantage of cloning

- Cloning is used by livestock producers to duplicate exceptional animals and utilise them as breeding stock to upgrade entire herds.
- Cloning is also used to produce high-quality livestock, which produces high-quality meat and meat products.
- Clones' first- or second-generation children may eventually end up in the food supply.
- Animals that have been cloned are safe for human consumption.
- Consumers, producers, animals, and the environment all gain from it.
- Cloning allows for the preservation and extension of proven, superior genetics.
- Meat-producing clones have solely traditional animal genes.

Problems associated with cloning

- Compromised immune system.
- Once clones used for breeding meat-producing animals can no longer reproduce.
- Cloned animals are now too expensive to employ in food items, costing upwards of $15,000 per animal.
- Currently, meat from cloned animals or their descendants is not sold to the general public. Because cloning animals is so expensive, only their progeny, not the animals themselves, would enter the food supply for human consumption.

Conclusion

In meat animals, meat quality is crucial for economic reasons. Multigenes and the environment are in charge of it. Advances in molecular genetics have led to the identification of genes, or markers associated with genes, that alter meat quality over the last few decades. Increased customer demand for high-quality meat promotes the development of technology to increase the quantity of high-quality meat tissue, which is why increasing the quantity of high-quality meat tissue is a long-term goal of the meat business. The main objective of genetics at the moment is to uncover elements in the molecular or biological components of meat quality that can be used to create "artificial meat" using genetic and molecular approaches. We know there is a lot of variation in muscle and meat characteristics between and within breeds and species, so traditional science has used breeding strategies like dominant trait selection and preferred trait selection by crossbreeding,

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as well as exogenous and endogenous hormones, to improve muscle and meat parameters. As a result, biotechnology is a novel approach to the procedures used to increase the quality of meat animals and meat. In this context, technology for various fake meats, such as meat from genetically modified organisms or cultured meat, may become sufficiently developed for these items to join the market, although competition across meat products is extremely difficult. As a result, biotechnology is a more severe scientific method that has the potential to increase the quality, yield, and safety of animal products by manipulating livestock genetically. However, progress in this area is gradual and there is still a long way to go before it has a commercial impact.

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