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Genetically engineered animals in future breeding programs

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Genetiskt modifierade djur i framtida avelsprogram

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Omslagsbild: Double muscled calves at Louth agricultural show, Ireland. Photo taken by Gro Hylén

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Abstract

The purpose of this literature review is to investigate what genetically engineered animals are being used for and if genome editing is going to be a useful tool in future animal breeding. Genetically engineered animals have been used within the biomedical research field since the seventies. The techniques of how to manipulate the genome of an organism has been developed from incorporations of transgenes via direct cytoplasmic microinjection into one-cell-stage embryos, to techniques that can change specific bases in the genome without adding transgenes. The most recent technology are referred to as genome editing and it uses engineered nucleases to target and change the bases of interest. This new method for the introduction of mutations is thought to revolutionize the field of livestock production. A few examples of genetically engineered animals are brought up in this review. Improvements made in the animals have been better food utilization, disease resistant traits or more environmentally friendly farm animals. However, the biggest advantage of using genetically engineered animals in livestock production lies within the biomedical research field, and it is believed to do so in the future as well.

Sammanfattning

Syftet med denna litteraturstudie var att studera vad genetiskt modifierade djur används till, hur de produceras och om den nya tekniken som kallas genome editing kan komma att vara ett användbart verktyg i framtida avelsprogram för att öka eller gynna animalieproduktionen. Genetiskt manipulerade djur har använts inom medicinsk forskning sedan sjuttiotalet. Genmodifieringsteknikerna har utvecklats från att inkorporera transgener via mikroinjektion direkt i embryos, som befinner sig i en-cell-stadiet, till tekniker som effektivt kan ändra specifika baser på genomet utan att addera transgener. De senaste teknikerna kallas för genome editing och de bygger på att specifika nukleaser lokaliserar och ändrar baser på genomet. Denna nya metod som används för att introducera förbättrande mutationer tros revolutionera animalieproduktionen. I den här review artikeln finns några exempel på genetiskt förändrade djur. Egenskaperna som förbättrats är bättre foderomvandlingsförmåga, bättre resistens mot sjukdomar eller miljöförbättrande egenskaper hos lantbruksdjur. Den största fördelen med att kunna använda genetiskt förändrade djur är inom medicinsk forskning, och det tros också vara så de närmast kommande åren.

Introduction

Scientists have since the seventies been able to manipulate the DNA of living organisms using biotechnology. The discovery of embryonic stem cells (ESC) and its ability to colonize the germline was first found in mice and ESC-mediated transgenesis became a useful tool for gene targeting in 1981 (Polejaeva *et al.*, 2015). The technique used to produce a transgenic animal back then was pronuclear microinjection (MI) of exogenous DNA directly into one-cell-stage embryos (Gordon *et al.*, 1980). The first genetically engineered farm animal was produced over 30 years ago by using this technique (Hammer *et al.*, 1985). Successful somatic cell nuclear transfer (SCNT) using an embryo-derived differentiated cell population resulted in the breakthrough for genetically engineered animals, Dolly the cloned sheep in 1996 (Campbell *et al.*, 1996). SCNT has since then been used to produce cloned cattle, goats and pigs (Laible *et al.*, 2015; Polejaeva *et al.*, 2015; Tan *et al.*, 2016).

Techniques have been further developed and new methods have been suggested, one of which is genome editing. Genome editing is based on engineered nucleases that can locate a specific target site at the genome. The nucleases induce a double stranded break (DSB) that are followed by one of the two repair mechanisms, the error prone non homologous end joining (NHEJ) or homology directed repair (HDR) (Gaj *et al.*, 2013). Edits of the genome is achieved in the repair process. HDR can introduce one or several transgenes if an engineered nuclease, together with a donor plasmid, bearing locus-specific arms, is co-delivered into an endogenous locus. To provoke mutations, insertions or deletions, single stranded DNA oligonucleotides and a linear donor sequences with <50 bp of homology can be used instead. The NHEJ mediated repair pathway after a DSB causes small insertions or deletions that results in knockout (KO) of gene function because of a frameshift mutation. (reviewed in Gaj *et al.*, 2013).

The engineered nucleases or designer nucleases, as they are also called, can be divided into two classes. The first one includes the zink finger nuclease (ZFN) and the transcription activator like effector nuclease (TALEN), both are modular protein containing a specific DNA binding domain and a catalytic cleavage domain consisting of the restriction enzyme fokI. The second group includes the meganucleases and the clustered regulatory interspaced palindromic repeat/CRISPR associated gene (CRISPR/Cas) system. The CRISPR/Cas system is increasing rapidly in livestock genetic research. (Reviewed in Sander & Joung, 2014; Jenko *et al.*, 2015; Tan *et al.*, 2016).

The first genome edited animal depended on modification of primary cells which were later used as nuclear donors for embryo reconstruction in SCNT (Hauschild *et al.*, 2011). This was followed by direct modification of the zygote through cytoplasmic injection (CPI) (Tan *et al.*, 2016). In the past five years more than 300 pigs, cattle, sheep and goats have been genome edited. The genome edited animals can potentially serve as organ donors, disease models, bioreactors or new lines of high producing or disease resistant farm animals. (Carroll & Charo, 2015; Tan *et al.*, 2016). The biggest advantage of producing genetically engineered animals lies within the biomedical field, but also within the farm animal industry (Laible *et*

al., 2015). So far only one transgenic animal is available for food consumption (AquaAdvantage® salmon) (Commissioner, 2016), even though many attempts have been made, for example to breed more environmentally friendly animals for meat production (Enviropig®) (Golovan *et al.*, 2001). This might change now as genome editing allows specific changes in the genome without leaving any traces of transgenes (foreign DNA) in the produced animal (Tan *et al.*, 2016).

The purpose of this literature review is to investigate what genetically engineered animals are being used for and if genome editing is going to be a useful tool in future animal breeding in the prevention of an upcoming food crisis in the world.

One of the promising techniques within the field of genome editing

The CRISPR-Cas system was found in bacteria and archaea and enables to detect and protect against mobile genetic elements. The system matches DNA-sequences from virus or plasmids and is believed to help the adaptive immune system to fight foreign genetic material. Genetic and biochemical experiments confirmed this hypothesis. (Jinek *et al.*, 2012). The CRISPR-Cas type II system uses an endonuclease, with the name Cas9 (Charpentier & Doudna, 2013). This enzyme can, together with a guide RNA, locate and splice foreign DNA at specific locations demarcated of conserved sequences called proto-spacer adjacent motifs (PAMs). To form the functional DNA-target complex, Cas9 needs two distinct RNA transcripts, CRISPR RNA (crRNA) and trance-acting CRISPR RNA (tracrRNA). The double RNA can configure into a single-guide RNA (sgRNA) that include a sequence which is adequate to program Cas9 to create a DSB in target DNA. (Jinek *et al.*, 2012; Charpentier & Doudna, 2013). The DSB is thereafter repaired by one of two repair pathways, NHEJ or HDR (Gaj *et al.*, 2013). Studies where Cas9 coding mRNA, together with a guide RNA, was injected into one-cell-stage embryos of zebrafish produced high frequencies (24-25%) of insertions and deletions (specific kinds of mutations) (Hwang *et al.*, 2013). This was interpreted as an indicator that the method can work in mammals and plants as well (Hwang *et al.*, 2013). Using the Cas9 enzyme has enabled modification of endogenous genes in organisms that before have been difficult to manipulate genetically (Sander & Joung, 2014).

Genetically engineered (GE) animals

Examples of recently produced farm animals created with the latest techniques

The basic method of how to produce a genome edited sheep using the CRISPR/Cas system together with CPI were described in Crispo *et al.*, (2015a). The ovaries from newly slaughtered sheep were recovered from the slaughterhouse and transported to the laboratory where the cumulus oocytes complexes were aspirated in recovery medium. The bulbous oocytes were fertilized with frozen-thawed semen. Cas9 mRNA and sgRNA were thereafter microinjected into the cytoplasm to induce a spontaneous mutation at a specific site. The aim of a specific case described in the study was to knock out the myostatin (MSTN) gene, which is a negative regulator of muscle growth. The zygotes were, after a couple of days of maturation in vitro, transferred into surrogate ewes that were synchronized to be on the same

day in the estrous cycle as the zygotes. Out of 53 grade one (successful developed) blastocysts inserted in 29 recipient ewes 22 lambs were born. Three of those died at delivery or within the first day after birth. Biopsies followed by PCR showed that nine live lambs and one of the dead lambs carried the desired mutation, among the living lambs carried eight a homozygous and two lambs a heterozygous version. The lambs with the homozygous mutation in the MSTN gene showed 60 days postpartum a significant difference in measured weight compared to the wild type lambs, the lambs carrying the mutation at both alleles were 20 to 30 % heavier than other lambs. (Crispo *et al.*, 2015a).

Liu *et al.,* 2014 produced genetically engineered cows that expressed human lysozome (hLYZ) in the milk. Lysozome is an antimicrobial protein that plays an important role in the defense against bacterial infections, and human lysosome has better antimicrobial properties than bovine lysosome (Liu *et al.*, 2014). The somatic cells used in the study were manipulated with ZFN together with SCNT, and out of 118 embryos inserted into recipient cows, five calves were born alive. The milk of the genome edited cows showed no significant difference in milk composition, when comparing fat, protein and lactose content with normal cows. However, when bacterial infection was induced by intra mammary infusion of *S. aureus, E. coli* or *S. agalactiae* in the mammary gland, 19 out of 20 glands in non-transgenic cows received infection but none of the genome edited cows did (Liu *et al.*, 2014). Scientists of the same group also produced cows expressing lysostaphin in the milk with the same method and the same purpose as for the hLYS cows. It is stated in the article that 5799 embryos were successfully reconstructed, 140 pregnancies were observed and 8 out of 14 borne calves were still alive one month after birth (Liu *et al.*, 2013).

Yu *et al.*, 2011 produced genome edited cows that didn't express beta-lactoglobulin (BLG) in the milk. BLG is a whey protein that causes milk protein allergies. The gene was knocked-out using the ZFN technique together with SCNT. Out of 995 reconstructed embryos 50 developed into pregnancies, and eight genome edited calves were born, but six of the calves died soon after birth (Yu *et al.*, 2011).

Luo et al., 2014 manipulated Chinese yellow cattle to achieve a knockout mutation of the MSTN gene using ZFN-mediated mutagenesis together with SCNT. Mutation of the MSTN gene occurs naturally in Belgian Blue and Piedmontese cattle at high frequencies and it increases the muscle mass in these breed by an average of 20 % (Ansay & Hanset, 1979; Kambadur *et al.*, 1997). In total 1336 embryos were reconstructed, some of them were inserted in cows, and it resulted in 35 pregnancies, and out of 18 born calves two calves did carry the mutated gene (Luo *et al.*, 2014).

Manipulation of several genes simultaneously was done by Ni et al., (2014), using the CRISPR/Cas9 method, who showed that it was possible to achieve mutations in four genes at the same time (MSTN, Prp, BLG and NUP). However, only the experiment with fibroblasts carrying mutation in the MSTN gene was used for SCNT and it resulted in the birth of two live goats from 21 transferred embryos (Ni *et al.*, 2014).

Zygotes from bigger mammals (pigs) were subject to manipulation with CPI for the first time in 2013 at the Roslin Institute in Edinburgh. In total 367 zygotes were injected with different concentration of RELA TALEN mRNA. The RELA gene was edited to cause resistance to the African swine fever, a viral infection that affects domestic pigs. After three days in vitro, 21 % of the embryos screened positive for edits in the RELA locus, and five live piglets were born which carried the porcine RELA mutation (Lillico *et al.*, 2013).

Whitworth *et al.,* (2014) tested if the CRISPR/Cas technique was as effective to produce edited pigs with CPI as with SCNT. They manipulated two genes, CD163 and CD1D, which are involved in porcine virus infections. A total of 2734 embryos with presumptive CD163 edit were produced with SCNT, and 34 edited piglets were born alive. Additionally 1055 embryos with presumptive CD1D edit were produced with SCNT, and 12 gene-edited piglets were born. A total of 96 embryos with presumptive CD163 edit produced with CPI, resulted in four live edited piglets. And finally, 110 embryos with presumptive CD1D produced with CPI, resulted in four live edited piglets (Whitworth *et al.*, 2014).

In 2015 the first genome edited sheep and cattle were produced using CPI together with TALEN mRNA, targeting a knockout of the MSTN gene. A total of 20 edited bovine embryos were transferred into 11 recipient cows, and it resulted in two full term twin pregnancies. However, only one of the pregnancies resulted in the birth of two calves, one heifer and one bull, and only the bull carried the mutation (Proudfoot *et al.*, 2015). The same experiment was done in sheep. From a total of 26 blastocysts transferred into nine recipient ewes 12 live lambs were born from eight successful pregnancies, although only one lamb carried the mutation (Proudfoot *et al.*, 2015).

The polled (hornless) trait is a desired trait in farmed cattle breed. Horned cattle can injure other animals in the herds or people working with the animals. Dehorning of cattle is associated with pain and stress for the animal and is also a costly procedure. The polled allele is naturally occurring in some cattle breed, for example the Angus. The trait can be introduced into horned cattle breeds by normal crossbreeding but unwanted effect like lower milk yield for example often follows as well. Tan et al., (2013) successfully introduced the polled allele into the genome of Holstein, a cattle breed that naturally inherits horn. The allele was introduces by a plasmid HDR template containing fragments from the Angus breed polled allele. Out of 226 colonies five screened positive for the introgression of the new allele. (Tan *et al.*, 2013).

A simulation study by Jenko et al., (2015) proposed a method of how to promote favorable alleles by genome editing. Animal breeding aims to increase the genetic merit of traits that are valuable in the production. These traits are often controlled by several quantitative traits nucleotide (QTN). Recent use of genomic selection (GS) associate phenotype with markers at the genome. This method speed up genetic gain, but with conventional breeding the frequencies of favorable alleles at the QTN will still be slow to achieve because of the low level of recombination during meiosis. Implementation of genome editing in breeding programs would allow individual alleles at QTN to be removed or added and this will rapidly

increase the genetic merit for valuable traits in livestock breeding. Ten simulations scenarios was performed where 25 sires was selected on the basis of their true breeding value (TBV) and then they were edited for various amounts of QTN (0-100), with the assumption of entirely additive QTN. A number of 1000 candidates were generated in each generation and 20 generations were counted for. The changes in frequencies of favorable alleles using only GS were 0.02 and 0.04 using GS combined with genome edited sires. The best results for cumulative response was when top five TBV sires were edited in 100 QTN, it resulted in a 4.12 times greater cumulative response than with only GS. (Jenko et al., 2015).

GE animals for commercial use

AquaAdvantage® salmon

The AquaAdvantage® salmon was first produced in 1989. It was produced by microinjecting a growth hormone (GH) transgene into the cytoplasm of a fertilized, non-water activated salmon eggs (Cook *et al.*, 2000). In September 1995 the company Aquabounty technologies applied for an investigation for future approval by the U.S. Food and Drug Administration (FDA) of their transgenic salmon (reviewed in Van Eenennaam & Muir, 2011). The new gene incorporated in the salmon that enhances growth is classified as a drug by the FDA, therefore it needs to undergo certain risk assessment. The salmon was finally approved to be safe to eat and to be sold for human consumption, in USA, in November 2015. The only production environment approved is land-based contained hatchery tanks, which are located in Canada and Panama. This aims to eliminate the risk of any salmon escaping into seawaters. As an additional precaution the fish are made reproductively sterile and are unable to breed in the wild. (Commissioner, 2016). The aqua advantage salmon contains rDNA that originates from the Chinook salmon, and the expression of this gene is under the control of an antifreeze protein promotor from an Ocean Put. The promotor turns the gene expression on and allows the GE salmon to grow faster (Medicine, 2016; Cook *et al.*, 2000; Van Eenennaam & Muir, 2011). A study by Cook et al., (2000) showed that the aqua advantage salmon had a 10 % better food utilization than the non-transgenic salmon and it reached the weight of 55 g four months earlier. According to the information on the website of Aquabounty, the transgenic salmon reaches a size of 4 kilo more than 200 days before the non-transgenic salmon (600 days vs 850 days) (*Technology, 2016*).

Enviropig®

The enviropig® was a transgenic pig firstly produced in 1999. The pigs were produced for the purpose of reducing phosphorus (P) leakage from the manure - with the aim to produce a more environmentally friendly farm animal. Pronuclear microinjection of the PSP/APPA transgene (parotid secretary protein promoter linked to the e-coli appA phytase gene) into embryos resulted in pigs which expressed phytase in their salivary gland and were able to digest phytate. The transgenic pig reduced the excreted phosphorus by up to 75 % (Golovan *et al.*, 2001). A study by Novoselova et al., (2013) evaluated the economic gain for farmers keeping the GE pig, regarding the effect of the Enviropig®. They could show that the costs of

a piglet produced were 2.5 % less than the non-enviropig, under the presumption of a stable meat price. The main reason for the cost reduction was that less P was required in the feed and manure cost was reduced due to fewer minerals in the manure (Novoselova *et al.*, 2013). Despite the good results, the funding's for the enviropig project reached an end in 2012 and all the remaining pigs had to be euthanized (Clark, 2015).

Glofish®

Not surprisingly, the Glofish® derive from zebrafish as the zebrafish is one of the most widely studied species within biological research. A protein expressing fluorescent color extracted from jellyfish or sea coral, was incorporated into the zebrafish to study cellular processes. Since the luminous color attracts the human eye, someone sought to sell them as pet fish. The Glofish® can now be purchased and kept in home aquarium in the US but they are banned elsewhere. The first glofish was first produced in Singapore 1999. (Davies, 2014).

Animals in biomedical research

It has been hypothesized that the latest techniques for manipulating the genome (genome editing) in farm animals increases the availability of livestock models for biomedical research. Mouse models have a long history as a model species within the research industry. In the past 25 years the mouse has contributed to the understanding of gene functions and regulations. The mouse is most often the first animal used for trials for clinical experiments. However, translating clinical trials from mice to humans has a low level of success rate. This is most likely due to the small body size and short lifespan of mice. Bigger animals such as cows, pigs, goats and sheep can replace their role in research and serve as better models for humans, especially when techniques for genome manipulations with higher success rates are developed (Polejaeva *et al.*, 2015).

Cattle (Bos taurus)

Cattle are an excellent model for human female reproduction and ovarian function as they have a similar reproduction cycle to women. Assisted reproductive technologies (ART) are used in humans and cattle. A higher risk of abortion and pregnancy problems due to abnormal placentation is associated with ART in women as well as cows (Polejaeva *et al.*, 2015).

Sheep (ovis aries)

Sheep are useful for studying the respiratory organs. Size of the lungs and breathing frequency is much like humans. A lot of research is being done for asthma and cystic fibrosis (Tebbutt *et al.*, 1995). Sheep are also commonly used as a model for pharmacological testing as certain brain structures are similar to that of human (Jacobsen *et al.*, 2010).

Goats (capris hircus)

Goats are used for research on atrial fibrillation, the most common serious abnormal heart rhythm. A trans-genetic goat that overexpresses the growth factor TGF-Beta1 has been breed

to be used as a model for atrial fibrillation. Goats are also used for investigations of mechanical circulatory support and a model for complete female-to-male XX sex reversal as a result of genomic mutation (Pannetier *et al.*, 2012; Polejaeva *et al.*, 2015).

Pigs (Sus scrofa)

Pigs are commonly used in biomedical research. They reach sexual maturity early in life, they breed all year around and they have large litter size, these traits make them into excellent research models. Many transgenic pigs have been produced to serve as models for human disorders and diseases (Polejaeva *et al.*, 2015). One core idea of the use of pigs is the idea of xenotransplanation, thus changing the animals to reduce organ rejection in human after transplantation (Dooldeniya & Warrens, 2003).

Discussion

In order to feed the growing population in the world new technology should be explored to increase the food production. Genetic modification of plants and farm animals is one possible way to achieve this (Crispo *et al.*, 2015b; Laible *et al.*, 2015). Incorporating genome editing in existing breeding program can speed up genetic gain that otherwise would take several generations. It would also reduce expenses both primarily and secondary. Primarily because less animals are needed for selection and secondary because achievable traits can add economic gain for the farmer (Novoselova *et al.*, 2013; Carroll & Charo, 2015; Jenko *et al.*, 2015).

Many GE animals have already been produced within the biomedical field but so far only one is available on the market for food consumption. The technique of genetically manipulating an organism was first applied to animals, but the technique has since been much more widely used within the plant and crop industry. There are a number of transgenic plants and crops out on the market in several different countries (Laible *et al.*, 2015). Transgenic plants seems to be easier for people to accept as a food source, additionally plants are easier to produce. The long reproduction cycles of animals and animal welfare concerns are obstacles to overcome in making GE animals a general success (Laible *et al.*, 2015). In the listed examples of recently produced GE animals, the success rate of getting live animals appears to be very low, yet, the authors of the reports put forward the technique as being a revolutionary technique, ready to be applied in animal breeding (Crispo *et al.*, 2015a; Liu *et al.*, 2013; Yu *et al.*, 2011; Whitworth *et al.*, 2014; Proudfoot *et al.*, 2015; Jenko *et al.*, 2015). Many of the difficulties are due to the fact that SCNT is a very difficult technique to master and the low percentage of successes (live born animals), is partly due to the massive amount of cloned somatic cells that are being cultivated but never used. The scientists safeguard themselves to get some progeny of the clones, or to be able to pick the most viable clones for transferring them into surrogates. Calculating the success rate (percentage of animals born alive) from the amount of inserted embryos is a better alternative to get accurate results. SCNT is highly associated with abortions and defects at birth, as well as early post-natal death. This adds to the relatively low live born rate and early death in the examples shown here. However, SCNT is a well-

established technique for cloning farm animals and so far 33 out of 43 successful genome edited animals has been done using SCNT (Tan *et al.*, 2016). On average, out of 130 reconstructed pig embryos transferred with SCNT, only one piglet is being born. To deal with the problems associated with SCNT, another technique, CPI, is starting to be more widely used. The technique of CPI is thought to be easier to manage than SCNT, but the success rate of getting live born animals is still low (pig 37% vs 76% for SCNT). However, CPI only requires an average of 24 pig embryos to achieve one live piglet (Tan *et al.*, 2016). The main aspect of using SCNT is that the newly introduced mutation can be detected before insertion in recipient surrogates and possible saving the labor of transferring supposedly edited embryos, in contrast to CPI where the edited animal cannot be identified until the birth. The positive effect of using CPI is that the zygotes that are being used, includes genomes from two parental gametes and thus the genetic variation is being preserved. Manipulation techniques using CPI is thought to be the favorable method to produce genome edited farm animals in the future (Ni *et al.*, 2014; Tan *et al.*, 2016).

What can the GE animals be used for

Bovine expressing hLYZ or lysostaphin in their mammary gland would be resistant to gram-positive bacteria like *S. aureus*. Having such genome edited cows in a dairy herd would be a big advantage since mastitis caused by *S. aureus* requires the use of antibiotic, which in turn will increase costs for the farmer (Liu *et al.*, 2014). The genome edited bovine will also have a general negative effect of antibiotic use. Another big advantage with transgenic dairy cows, would be the opportunity to manipulate the genome so that they produce milk that is more similar to human milk. The milk would be an excellent infant formula or would allow people with allergies to normal milk to consume milk. Concerning meat, the traits of interest often lies within producing more meat at a lower cost. This requires the breeding of animals with better food utilizations traits. One way of achieving this is by causing a mutation in the MSTN gene. The MSTN gene is well recognized and easy to target for scientists. Another way to achieve increased growth is to insert a transgene including a growth factor hormone, like in the example with the salmon. But maybe more interesting would be to produce farm animals with healthier meat, containing more essential fatty acids (omega 3) (Laible *et al.*, 2015). The examples of application of GE for the production of healthier and more disease resistant herds are possibly the areas which would be most accepted by the general public. Lethal mutations could be eliminated or treated with GE. Lillico et al., (2013) produced genome edited pigs, with edits in the porcine RELA gene, with the aim to produce pigs resistance to the African swine fever. The viral infection affects domestic pigs but African pigs such as warthogs appear to be resistant to the virus. Producing animals that are more environmentally friendly, is another important topic and a major concern for future food production. The Enviropig® that reduced its P output with up to 75 % could have been a huge success (Golovan *et al.*, 2001). Breeding or producing transgenic animals that are serving a purpose of a pet (Glofish®) might, however, not make much sense.

When applying the most recent developed techniques of genome editing summarized in this review (ZNF, TALEN and CRISPR) it is possible to manipulate specific bases of single genes

in the genome of farm animals with high precision. However, the legislation has yet to decide on the classification of these techniques. The genome edited animals would under most of the current legislation, likely not be classified as transgenic animals. The edits made would rather been seen as normal mutations that sometimes occur in the wild, like a mutation in the MSTN gene that already occurs naturally in some beef cattle breeds. The benefits of using genome editing in animal breeding are that genetic gain can be reached faster than with only selective breeding. The new techniques are thought to revolutionize the biomedical research field as well as solving the need for expanding food production for a growing population. Incorporating animal within the biomedical field sees endless opportunities. Among some examples, cows and goats can be used as bioreactors to produce certain substances in their milk and pigs can be used as organ donors. Medicines will also be customized to suit individual genome with genome editing techniques (Gaj *et al.*, 2013; Sander & Joung, 2014).

Negative aspects of GE

Genome editing is not yet approved by governments, which is the first holdback to apply the technique widely in animal breeding. The desired traits are often controlled by several quantitative trait nucleotides (QTN) and it can be hard to exactly know which gene controls which trait, as well as to predict underlying epistatic effects. To be able to edit the genome, favorable alleles will need to be identified by associating genotypes with corresponding phenotypes. With genomic selection (GS) being more popular within farm animal breeding it is thought to create a large dataset with needed information to implement GE in a safe and well controlled manor (Jenko *et al.*, 2015).

People that are in opposition to genetically engineered animals argue that it will disturb the biological diversity, especially if edited individuals breed with their wild type relatives. That is the reason why genetically engineered animals need to be contained in well secured areas. That is also partly the reason why genetically engineered animals are so far very expensive to keep. Critics called the Enviropig® the frankenswine and refused to accept the facts that it was a normal pig with the exception of expressing phytase in the saliva. However, the supporters of genetically engineered organisms persist in saying humans have since the first day of domestication, may it be a plant or an animal, manipulated the development of the organisms. It just takes one look at the beef cattle production to realize that the theory might be right. The Belgian Blue, for example, that naturally inherits the knockout of gene function in the MSTN gene, expresses double muscling. It has been produced by conventional breeding strategies, and it is a proof that the genome has a wide variety to be manipulated only by selective breeding. The question remains whether it is ethical to produce such animals.

Conclusion

Manipulation techniques have so far been used to produce fast growing animals with better food utilization traits, disease resistant animals, healthier animal-derived products, environmentally friendly animals, pet animals and animals serving a purpose in the biomedical research field. Genome editing techniques has not yet been implemented in

breeding programs for farm animals. The only reasonable method of incorporating genome editing in existing breeding programs would be to introduce few males with edited genome, like suggested in the simulation study of Jenko et al., (2015). In the near future it is most likely only going to be used within the biomedical field. The negative aspects of using genome editing in animal breeding is the low level of success rate in producing live animals.

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