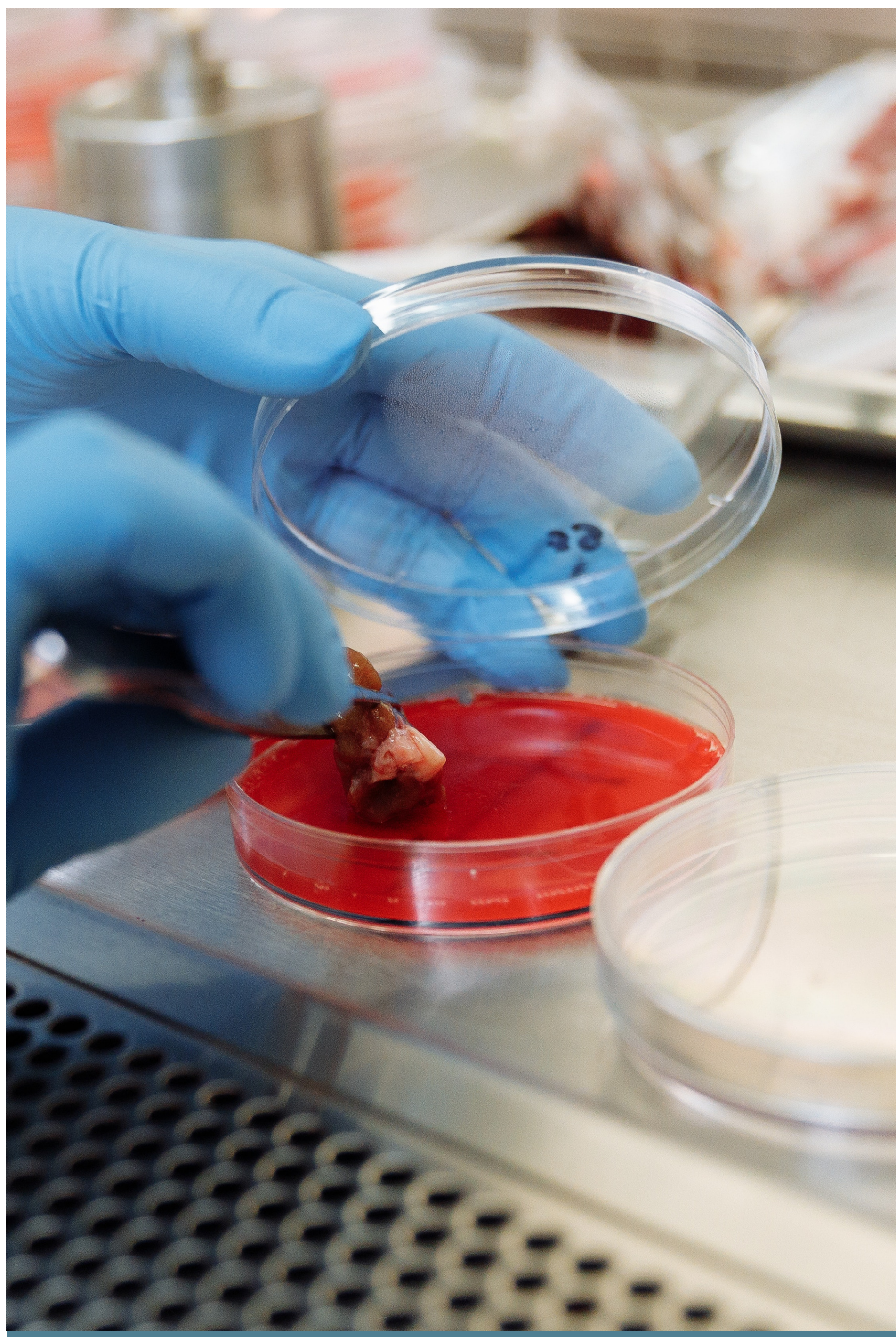
CRISPR/CAS9: THE FUTURE OF MEAT & DAIRY

THE EXCITING MOVE FROM PASTURE TO LAB

"Cultured meat is going to change the world," Daan Luining, Founder and CTO, Meatable



CRISPR/Cas 9 from the microbial adaptive immune system facilitates a pioneering method of genome engineering and has revolutionised the world of genetics. This system has made it faster, cheaper, more efficient and more accessible than ever to readily alter genetic material. The ability to engineer biological systems and organisms holds enormous potential for applications across basic science, medicine and biotechnology, but this now includes food. Food has been pivotal in society since the beginning of time and is now being reimagined entirely through CRISPR/Cas9.

The world has rapidly grown in the past few decades, and with it the need for accessible, rapid, large quantities of meat and dairy production. Animal products make up a large majority of food consumed in Western diets. (11) There are however several issues with modern food consumption and food production processes. Protracted and persistent environmental and social deterioration brought on by the continued high consumption of animal products worldwide has led to climate change, biodiversity loss, water stress, and water pollution.

CRISPR/Cas9 can help address these issues by providing a means for faster, more efficient, less allergenic, and more sustainable meat and dairy output.

"It's going to change how we view meat, how food is produced, what type of food is being produced, where it's going to be produced, and what people will demand of it — on quality, on texture, on taste, on nutrients." Daan Luining.

CRISPR DAIRY:

Dairy is arguably one of the most culturally significant foods worldwide. In the United States the average person consumes 297.1 kg of dairy products per year (16). Although dairy alternatives exist, few have been a match for the characteristic flavour and nutritional profile of animal dairy. Dairy is one of the largest contributors to greenhouse gasses and deforestation, making finding a suitable replacement a necessity. Enter CRISPR Milk.

In terms of flavour and nutritional value, synthetic milk is quite similar to cows' milk, but it is produced without using any actual animals. Instead, the crucial dairy proteins for flavour and texture are artificially created in a lab away from any pastures where herds graze. When compared to milks made from nuts and plants, it closely matches the taste and consistency of cows' milk and is similar to cow's milk in terms of nutrient profile. Since no cows were used in the creation of this milk, it is also suitable for vegans. For people who are lactose intolerant, synthetic milk is also an option. Lab milk can be created without lactose and it won't give lactose-intolerant people the same reaction that cow's milk does. The manufacturing method is drastically more environmentally friendly than dairy farming since there are no animals to maintain, less waste products like manure and no vast land requirements for pasture or feed production. As there are no longer any need for animals, it is also more ethical.

Milk's protein is constituted by casein and whey. There are four main proteins in the casein family found in milk; each being relatively small and requiring no complicated 3D folding. Whey, is a definition used for milk's non-casein proteins, 90% of which is mostly made up of two proteins: alphalactalbumin, which regulates lactose synthesis in cows and is responsible for most of milks characteristic flavour profile; and beta-lactoglobulin. The remaining 10 per cent of whey consists of blood serum proteins and antibodies (3).

HOW?

Each of the key milk proteins are well characterised and have a known amino acid sequence which is easily converted into a DNA sequence (4). This can then be inserted into yeast and bacteria using a CRISPR/Cas9 system, similar to how gene insertion is applied in therapeutic protein production such as in insulin (5). Subsequent to protein synthesis in these cell types, all that remains is to combine these proteins with water, sugars and fats from plant-based oils with small, aromatic fatty acids, leaving the final product, economical and environmentally friendly milk.

CRISPR MEAT:

An exponential explosion of meat alternatives has hit the market in the past 10 years but as with alternative dairy options, many still fall below expectations. It's very difficult to replicate the texture, flavour and nutritional content of meat, so why try reinvent the wheel? Cultivating meat with CRISPR/Cas9 has the potential to revolutionise meat production.

Cultivated meat, also known as cultured meat, is genuine animal meat that includes seafood and organ meats, that is produced by cultivating animal cells directly. This form of manufacturing does away with the necessity to breed animals for food. Because the cell types in cultured meat are the same or close to those in animal tissues, the sensory and nutrient profiles of traditional meat can be accurately reproduced. The field of cultured meat was developed after years of research in cell culture, stem cell biology, tissue engineering, fermentation, and chemical and bioprocess engineering. A revolution in the meat and dairy industry is here, as research across these fields is being done by hundreds of businesses and academic institutions throughout the world in an effort to create a new paradigm for producing commodity meat products on an industrial scale.

Commercial production is anticipated to be achieved without the use of antibiotics which also aids in tackling the worlds' overuse of these drugs. Since there is no risk of exposure to enteric bacteria, there should be fewer instances of foodborne diseases. The $1.7 trillion traditional meat and seafood sector is anticipated to lose considerable market share over the next few decades to cultured meat and other alternative proteins. This change will reduce industrialized animal slaughter, antibiotic resistance, zoonotic disease outbreaks, and deforestation caused by agriculture (14). It also removes any concern with animal cruelty and welfare.

CULTIVATION PROCESS

The collection and storage of an animal's stem cells marks the beginning of the manufacturing process. Then, these cells are expanded at high densities and volumes in bioreactors. The cells are fed an oxygen-rich cell culture medium that contains essential nutrients including amino acids, glucose, vitamins, and inorganic salts, and is supplemented with proteins and other growth factors, much like what occurs within an animal's body. Immature cells are prompted to differentiate into the skeletal muscle, fat, and connective tissues that make up meat by changes in the medium's composition, frequently in conjunction with cues from a scaffolding structure.

After that, differentiated cells are collected, processed, and assembled into finished goods. Depending on the type of meat being grown, this procedure usually takes 2 to 8 weeks. (15)

Cultivated meat offers a number of advantages over traditional animal agriculture due to its more effective production process, including utilizing much less land and water, emitting significantly fewer greenhouse gases, reducing pollution and eutrophication associated with agriculture; and eliminating the need for animals therefore being more ethical. (15)

CRISPR/Cas9 can hasten the creation of cell lines appropriate for growing meat. Engineering cell lines can have a significant impact on final product qualities like nutrition or the productivity or efficiency of the production process. Genetic engineering or adaptation can both be used to develop cell lines. A cell line must be serially sub-cultured under various conditions and subjected to selection over time in order to adapt. A cell line produced through this procedure exhibits a novel characteristic or is suited to a new set of environmental factors. (15) Adaption to suspension growth or serum-free media are two common types of adaptation. However, the main area of interest for genetic alteration lies within nutritional modification of meat and growth efficiency.
One example of genetically modifying meat in vitro is nutrient fortification. Stout et al successfully engineered bovine cells to produce lab-grown beef containing beta-carotene, a plant nutrient that is converted into vitamin A in the human body. The researchers proposed the idea of using CRISPR/Cas 9 to nutritionally engineer meat to convey a broad assortment of health benefits not seen in regular farmed meat (1).

Another example is by modifying myostatin. Myostatin is a negative regulator of skeletal muscle mass. By knocking this gene out, it offers a strategy for promoting muscle growth. Yalin et al used CRISPR/Cas9 with in vivo models and successfully doubled muscle content while reducing fat content. This offers higher efficacy in cultivation as more “energy” is put into developing meat cells rather than expending it on fat generation, whilst also providing a leaner, lower calorie end product (2).

CONCLUDING REMARKS:

The world population is continuously growing, and with growth comes demand for resources such as meat and dairy. How we farm and produce food has serious detrimental effects on our population, culture, local environment, climate and biodiversity. The agricultural arena needs to adapt. Genetic engineering is emerging to become the leader in agricultural evolution and CRISPR/Cas9 has the flexibility, accessibility and versatility to bring about this change.

REFERENCES:

1. Andrew J. Stout, Addison B. Mirliani, Erin L. Soule-Albridge, Julian M. Cohen, David L. Kaplan,Engineering carotenoid production in mammalian cells for nutritionally enhanced cell-cultured foods, Metabolic Engineering, Volume 62,2020,Pages 126-137,ISSN 1096-7176, https://doi.org/10.1016/j.ymben.2020.07.011.

2. Zheng Yalin, Zhang Yu, Wu Liyan, Riaz Hasan, Li Zhipeng, Shi Deshun, Rehman Saif ur, Liu Qingyou, Cui Kuiqing. Generation of Heritable Prominent Double Muscle Buttock Rabbits via Novel Site Editing of Myostatin Gene Using CRISPR/Cas9 System. Frontiers in Veterinary Science VOLUME 9, 2022. 10.3389/fvets.2022.842074

3.Fox, P.F., Guinee, T.P., Cogan, T.M., McSweeney, P.L.H. (2017). Chemistry of Milk Constituents. In: Fundamentals of Cheese Science. Springer, Boston, MA. https://doi.org/10.1007/978-1-4899-7681-9_4

4.S.M. Rutherfurd, P.J. Moughan, The Digestible Amino Acid Composition of Several Milk Proteins: Application of a New Bioassay,Journal of Dairy Science, Volume 81, Issue 4,1998, Pages 909-917, ISSN 0022-0302, https://doi.org/10.3168/jds.S0022-0302(98)75650-4.

5. Khan, Ashraf & Ullah, Zabi. (2021). Using CRISPR-Cas9 for Therapeutic Protein Production (Review Article). Asian Journal of Pharmacy, Nursing and Medical Sciences. 9. 10.24203/ajpnms.v9i1.6115.

6. FAOSTAT, FAO Statistical Databases (Rome, Italy: 2014), http://faostat3.fao.org/faostat-gateway/go/to/home/E (accessed June 19, 2022).

7. FAO, Livestock's Long Shadow. Environmental Issues and Options (Rome, Italy: Food and Agriculture Organization of the United Nations, 2006).

8. M. Lovera, Meat from a landscape under threat: Testimonies of the impacts of unsustainable livestock and soybean production in Paraguay, Global Forest Coalition and Brighter Green (accessed June 19, 2022)

9. WWF, Living Planet Report 2014. Species and Spaces, People and Places(Gland, Switzerland: 2014).

10. Susanne Stoll-Kleemann & Tim O'Riordan (2015) The Sustainability Challenges of Our Meat and Dairy Diets, Environment: Science and Policy for Sustainable Development, 57:3, 34-48,DOI: 10.1080/00139157.2015.1025644

11. Henk Westhoek, Jan Peter Lesschen, Trudy Rood, Susanne Wagner, Alessandra De Marco, Donal Murphy-Bokern, Adrian Leip, Hans van Grinsven, Mark A. Sutton, Oene Oenema,Food choices, health and environment: Effects of cutting Europe's meat and dairy intake,Global Environmental Change,Volume 26, 2014,Pages 196-205,ISSN 0959-3780,https://doi.org/10.1016/j.gloenvcha.2014.02.004.

12. L. Bouwman, K.K. Goldewijk, K.W. Van Der Hoek, A.H.W. Beusen, D.P. Van Vuuren, J. Willems, M.C. Rufino, E. Stehfest. Exploring global changes in nitrogen and phosphorus cycles in agriculture induced by livestock production over the 1900-2050 period. Proceedings of the National Academy of Sciences, 110 (2013), pp. 20882-20887

13. P. J. Gerber et al., Tackling Climate Change Through Livestock. A Global Assessment of Emissions and Mitigation Opportunities (Rome, Italy: Food and Agriculture Organization of the United Nations, 2013).

14. Post, M.J., Levenberg, S., Kaplan, D.L. et al. Scientific, sustainability and regulatory challenges of cultured meat. Nat Food 1, 403–415 (2020). https://doi.org/10.1038/s43016-020-0112-z

15. Sharma, S., Thind, S.S. & Kaur, A. In vitro meat production system: why and how?. J Food Sci Technol 52, 7599–7607 (2015). https://doi.org/10.1007/s13197-015-1972-3

16. DFA. 2022. U.S. Dairy Consumption Beats Expectations in 2020 and Continues to Surge Upward Despite Disruption Caused by Pandemic. [online] Available at: <https://www.idfa.org/news/u-s-dairy-consumption-beats-expectations-in-2020-and-continues-to-surge-upward-despite-disruption-caused-by-pandemic> [Accessed 30 June 2022].