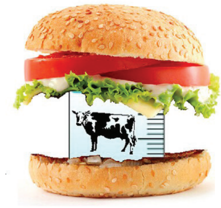
Securecell

Application note #009

November 2020



Next Generation Meat

Rethink the way of eating

Virginia Mello: Securecell AG, In der Luberzen 29, CH-8902 Urdorf, Switzerland (virginia.mello@securecell.ch)

The world population is expected to reach 9.6 billion people by 2050, the middle class is growing, and meat consumption is expected to increase by 70%. Meat production requires a great amount of resources: 34 kgs of feed are necessary to produce 1 kg of meat, the same kg of meat requires ca. 14000 l of water to be produced. (1)

Currently, growing meat in a conventional way consumes about 30% of global ice-free land, 8% of total freshwater, and produces 18% of global greenhouse gas emission. (2)

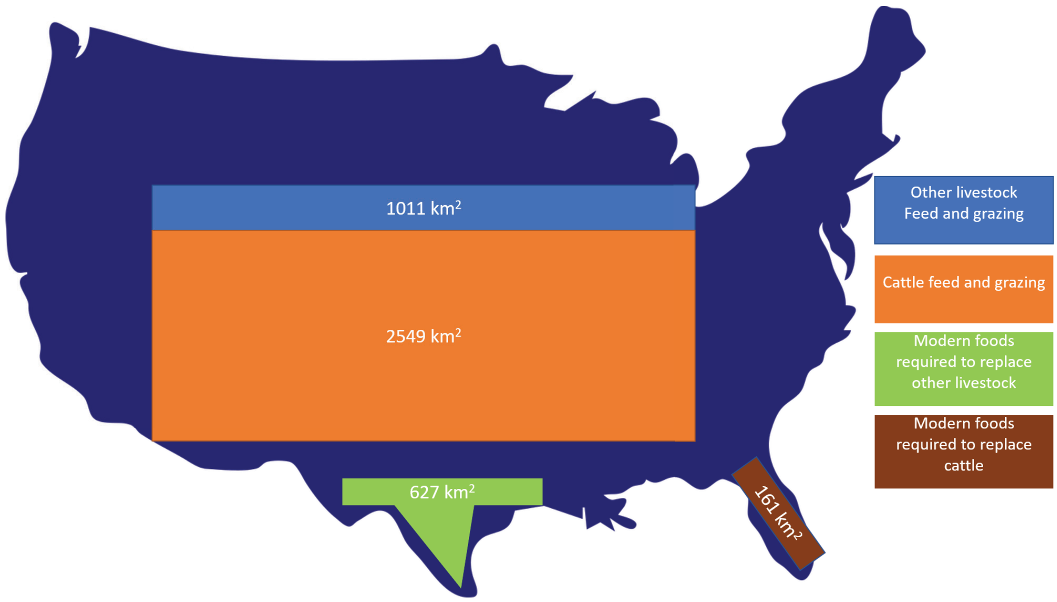


Figure 1: Land Acreage of Continental U.S.A. and its usage. Source: RetinkX

Moreover, ethical issues such as animal welfare and slaughtering are involved in meat production. Intensive factory farming and poor animal health are primary causes of the spillover of viruses that result in possible pandemics such as the Covid-19 and avian influenza ones, in addition to the spreading of E. coli, salmonella, and campylobacter that can be found in the meat. Each year in the USA, 48 million people get sick from contaminated food. 70-80% of the total annual use of antibiotics in the United States are used for farm animals, with the possibility of increasing antibiotic resistance, one of the major concerns of our times. Different breeds of animals are selected, and complex feeding protocols are developed to obtain meat with high culinary standards, requiring time and money. (3)

However, meat is an important source of nutrients for humans, such as the complex heme and iron, that is the only form of iron that humans can adsorb. Besides, most of the population is simply meat-eater.

In the last decades, more than 25 companies have been founded in order to find a solution for the meat production problem and arrive at a sustainable way of eating meat. The two major options are plant-based meat and clean-meat, a lab-produced meat.

The plant-based or "fake" meat

Companies such as Beyond Meat or Impossible Foods are trying to use plant-proteins to create food that resembles normal meat with a potential market of $ 450 billion by 2040. (4) The production involves mixing several ingredients to reach the perfect combination of ingredients to fully resemble meat. Plant-based meat could reduce the impact on the environment but there are still doubts about the question whether it is healthier than an equilibrate diet that includes a balanced intake of meat.

Clean meat

Clean meat is real meat. This name has been coined to avoid using the term "synthetic" because clean meat is not synthetic. The difference between meat and clean meat is that the second one is produced in a lab without slaughtering animals.

The process to produce clean meat is still not straight forward: it is inefficient, expensive, and requires extreme expertise. However, it could be a sustainable solution to feed the future population with meat without impacting the environment as much as with animal farming.

The production of clean meat has the potential to use 98% less water and emit 96% fewer greenhouse gases compared to the one of traditional meat. Clean meat does not contain artificial growth hormones, it does not enter in contact with bacteria or other contaminants such as Salmonella, on the contrary, it is cultivated in a sterile environment, it is not GMO and it does not contain antibiotics. (5)

In the last decade, tissue engineering and stem cell based-research experienced a boom in technology that drove to innovations such as the production of in vitro organs.

The same techniques can be applied to the production of clean meat: if engineered tissues are optimized for biological functionality, improved post-transplantation viability, biodegradability, and negligible immune response, clean meat is optimized in terms of flavor, texture, cost, nutritional value, and food safety.

Gene expression, metabolomics, and proteomics can be fundamental factors for meat flavor, quality, and safety assessments.

The flavor is fundamental in clean meat production: thousands of water-soluble and fat-derived molecules, which play an important role in giving the classical meat its tastiness, must be analyzed.

Quality is usually assessed through light diffraction; however, it is standardized on bigger samples of meat. Chromatographic separation and mass spectrometry are good, but expensive test methods to identify the molecular components of clean meat.

Texture can be analyzed through state-of-the-art devices, but it could be a problem to maintain this though the scalability process. Metabolic processes (anaerobic glycolysis, lactic acid accumulation, decreased pH, protein denaturation, and enzymatic proteolysis) influence the texture, appearance, and taste of the meat and comparable processes may occur in cultured meat after harvest. (6)

All those parameters must be controlled during the entire development process. A centralized bioprocess software, such as **Lucullus® PIMS**, could automatically control the process, produce data to scale up the process step by step and collect all the produced data, all in one solution.

Three different types of cells can be cultured to produce clean meat: stem cells such as bovine embryonic stem cells or mesenchymal stem cells that can differentiate into many different cell types, but are difficult to obtain. Satellite stem cells or adult stem cells, found in skeletal muscle tissue, are physiologically the cells that repair muscles after an injury. However, also satellite stem cells are difficult to obtain. The third option are fibroblasts and myofibroblasts, fully differentiated cells, that are easy to isolate, but difficult to propagate because of their short life cycle. Those cells are isolated from the animal through a non-dangerous muscular biopsy while embryonic stem cells are derived from the blastocyst stadium of the animal embryo.

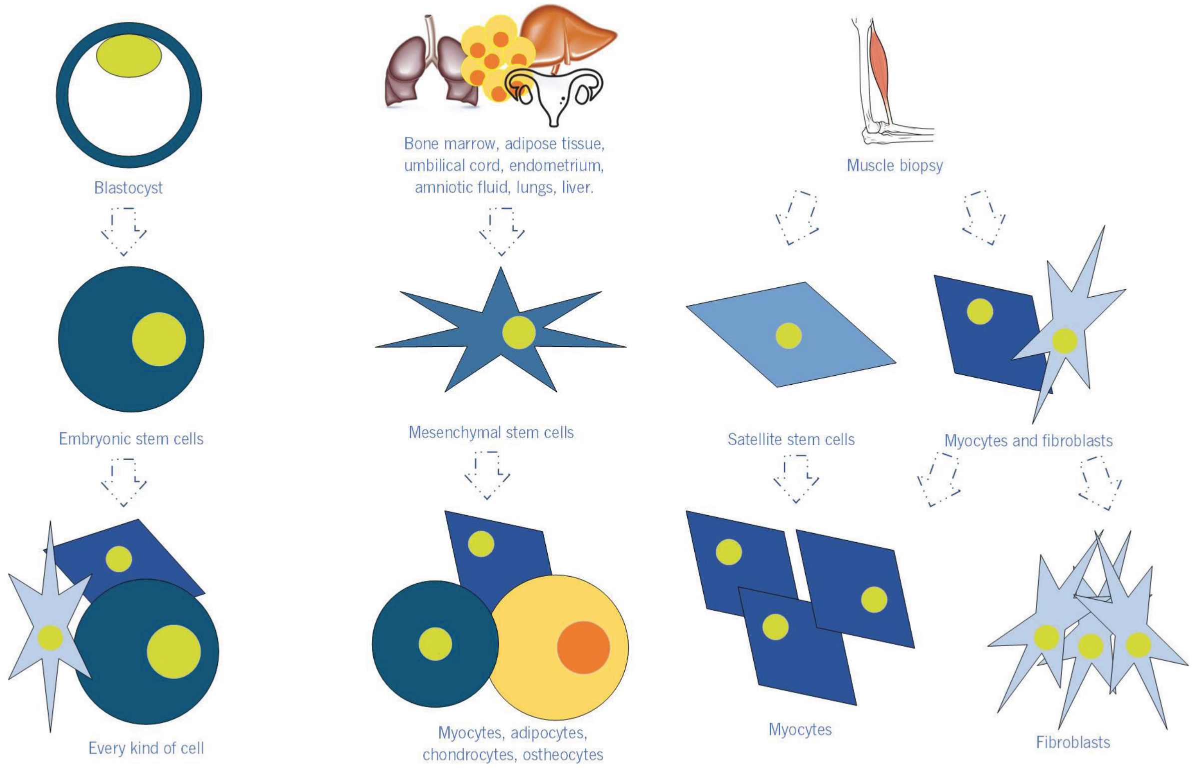


Figure 2: Cells that can be used for clean meat production.

The first step after obtaining the desired cells is to expand them. This step can be performed in shake flasks that allow the few cells to expand and obtain all the necessary nutrients. Lucullus® PIMS allows the automatic control of this process: it calculates the expanding volumes based on analytical data, controls at the same time all the different processes even with different parameters (volume, cell concentration, feed control, etc.), and is able to calculate the necessary steps and adjustments to scale up the production.

The second step of clean meat production is to expand the cells in a bioreactor: to obtain this, the bioreactor must act as a circulatory system in which nutrients and oxygen are fed and products of metabolism removed. The **Sephara** technology, the latest addition to Securecell's portfolio, boasts a wide set of defined pore size membranes that can be used for continuous bioprocessing and filtration. Sephara could be applied to clean meat production to ease the media recycling, eliminate the dead cells and residuals to maintain the perfect growth environment.

Tuomisto and de Mattos (2011) showed how having a recycling mechanism to refresh the media eliminating metabolism products such as carbon dioxide and lactate and maintain the nutrients constant would strongly benefit the cultures. Moreover, in combination with Lucullus®, **Numera®**, an automated sampling, and sample processing system, allows automating the workflow, including the sampling and sample preparation, having real-time analytical results, and a fast control over the perturbations of the system. Those devices will enhance the possibilities of process control while reducing the cost of production and obtaining a bigger and faster meat harvest.

The cultured cells can be positioned onto collagen-based hydrogel or gel cylinders where they can develop into contractile units. On the physical support, they are further stimulated to increase muscle protein, such as heme-carrying protein Myoglobin, produced with electrical stimuli, or physical stretch. Moreover, a constant automated sampling would allow controlling nutrients perturbations, changes in nutrient mixtures to obtain different products, such as adipocytes in the same mixture, and the biochemical composition of the meat at all stages.

Scaling up those processes is a challenge. Cell culture is not an efficient process and the media in which the cells are cultured are extremely expensive: 80% of the cost of production is due to the growth factors. Cost-effective systems to empower the process are necessary. Recycling some parts of the media and using lower-cost nutrients will reduce the cost of production. Moreover, Lucullus® offers many other advantages to the scaling up of the process: configuration of setup and step-chain operations for small bioreactors, where CPPs and operations can be calculated in parallel to be applied to scaled-up processes; scale down model based on real data; applicability of process to different devices: possibility to apply Lucullus® and the process to different environments with different devices; automatic unit conversions to keep data consistency and standardization.

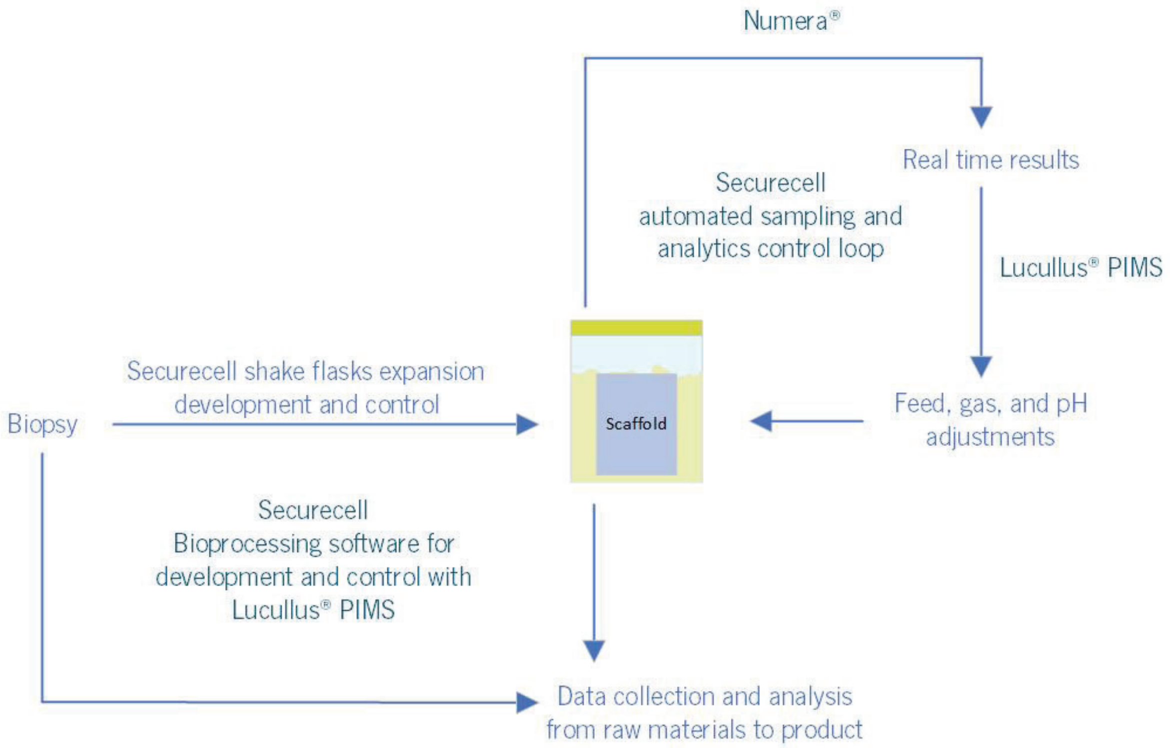


Figure 3: Closed control loop with Securecell devices.

A major problem is the shape of meat: a meatball or a burger is “easy” to assemble, but what about a steak? It has blood vessels, muscle, and fat. Meat contains about 90% muscle fibers, 10% connective and fat tissue, and 0.3% blood. Aleph Farms created the world’s first cell-based steak at the end of 2018 and in 2019 they successfully 3D printed meat at the International Space Station. (7) To obtain more structured pieces of meat, the cells can be cultivated on 3D scaffolds in a continuously perfused environment, where they can be co-cultured with fat and other types of cells. Even in a more sophisticated set up like the 3D culture environment, Numera®, Lucullus®, and Sephara will be extremely advantageous to increase the process performance. (8) However, ground meat is 40-60% of the output of a cow volume and can be used in a wide variety of ways. (9)

Many factors influence the meat price: Feed and hay, supplies, repairs, fertilizers, machines, animal costs, insurance, veterinary, taxes, breeding costs, etc. are direct costs difficult to be cut down. Especially in countries like Switzerland where the cost of machines and the skilled labor are extremely expensive. Now, 1kg of live cattle is traded at the CME (Chicago mercantile exchange) at about 1 USD and 1kg of pig (lean hog) 0.5 USD, the cost of packaging and production excluded. Assuming 25% of beef is eatable, this means an average trading price of 4 UDS/kg of beef meat. Intensive farming led to a decrease in the price of meat, allowing more access to it. However, this way of farming is unsustainable as discussed before.

To estimate the price of production of clean meat, we must start from the raw material: cells. Different beef breeds grow at a different rate and different cells at different differentiation stages also grow at a different rate. In average, before reaching the growth plateau, cells duplicate every 24h. One kg of muscle is estimated to be composed by 8 billion of cells. Starting a cultivation with 500.000 viable cells (average of available cells in a skeletal muscle cryopreserved vial) to obtain 1kg of meat, it will take about 10.5 days ($t = \ln(N/N0)/\ln 2$). This would mean a theoretical productivity in a perfusion system of about 50% of the productivity (mass x time) of a living cattle.

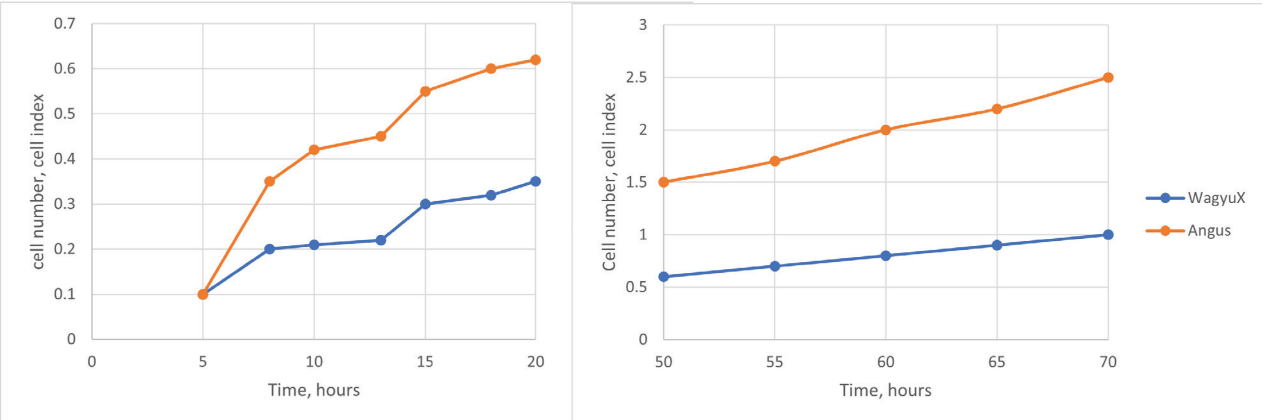


Figure 4: Figurative example on how Angus primary muscle cells grow faster than primary skeletal muscle cells derived from Hereford and WagyuX cattle. (10)

Future Meat Technologies, an Israel based start-up, plans to launch by 2022 clean meat that will cost less than 22 USD per 1kg. By 2030, a 70% reduction in the market for animal-derived ground beef is expected in the USA, which will be exchanged with precision fermented meat (or plant-based meat) and clean meat. (9)

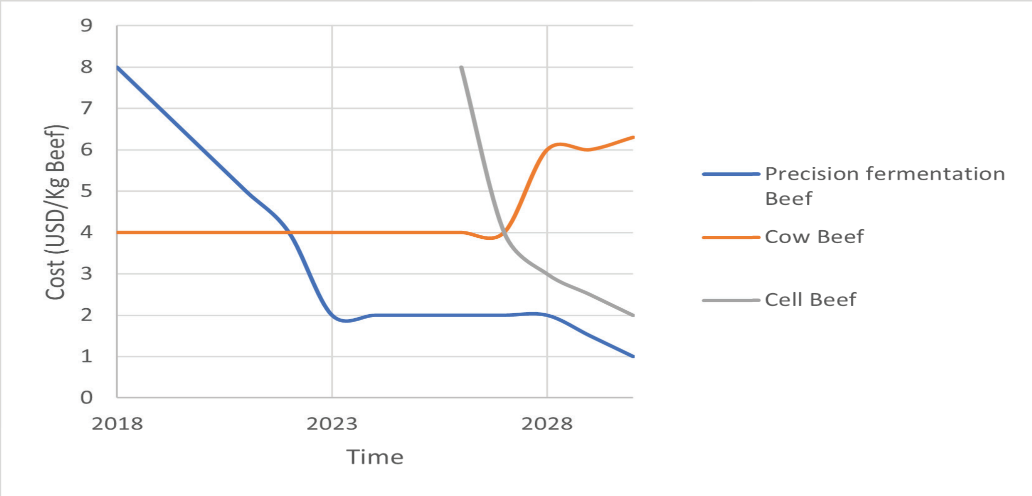


Figure 5: The cost curve for beef Source RetinkX

Securecell's portfolio

Securecell offers a complete range of products to close the loop in bioprocess development, monitoring, and control.

Lucullus®, a process information management system (PIMS), acts as a central data hub communicating with a vast range of devices to automate the production with ease. All data, including those from sample analytics and media preparation, can be automatically aligned.

In combination with Lucullus®, Numera®, an automated sampling, and sample processing system, allows automating the workflow, obtaining real-time analytical results, and accessing and controlling the processes at all time, even from remote. Thanks to Numera®, samples can be diluted or filtrated based on the analytical necessities or stored at 4°C.

Sephara is a probe with defined pore size (0.2-32 um) silicon membranes for bioprocessing pre-filtration or continuous perfusion. The Sephara technology can be applied to continuous processes to recycle the media while eliminating cell residuals and dead cells avoiding shear stress and assuring continuous performance avoiding fouling and clogging. With Sephara, a 0.5 l bioreactor could have a complete change of media 3 times a day.

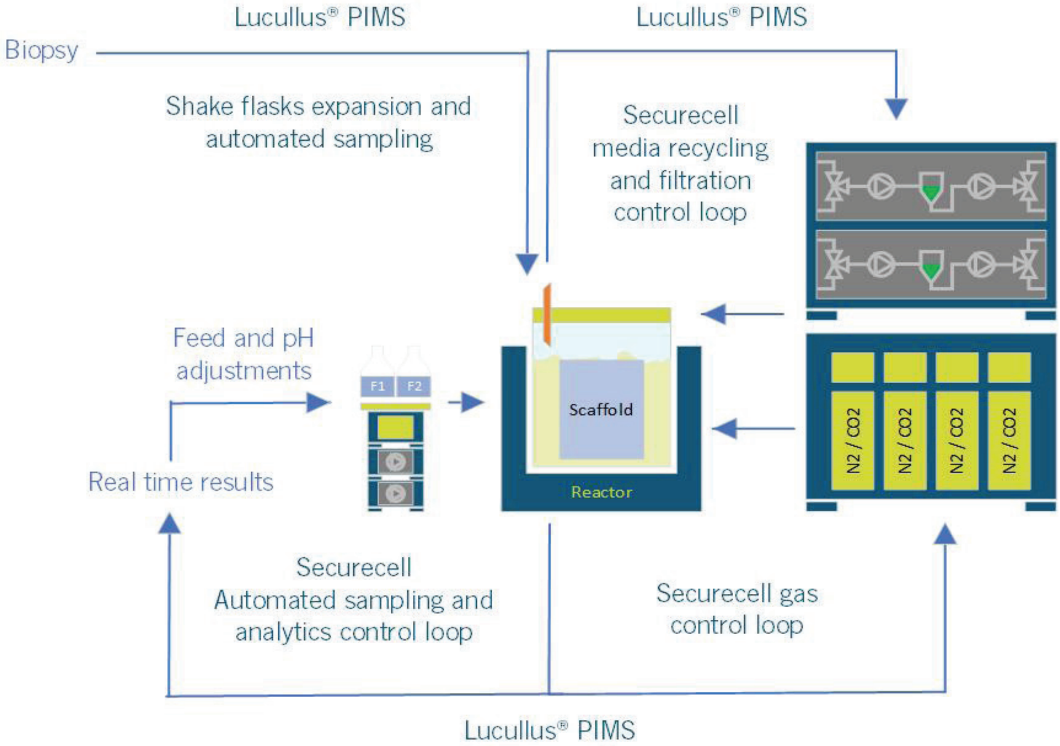


Figure 7: The future of lab automation with Securecell pipeline devices.

CONTACT

For more information
on our products and services
visit our website at

www.securecell.ch

Securecell AG
In der Luberzen 29
CH-8902 Urdorf, Switzerland

+41 44 732 90 70
info@securecell.ch

References

1. [Online] https://www.cnbc.com/2018/03/23/bill-gates-and-richard-branson-bet-on-lab-grown-meat-startup.html.

2. [Online] https://www.aocs.org/stay-informed/inform-magazine/featured-articles/clean-meat-february-2018?SSO=True#:~:text=Potential%20benefits%20of%20clean%20meat,structured%20meats%2C%20and%20adding%20fat.

3. [Online] https://www.frontiersin.org/articles/10.3389/fsufs.2019.00046/full.

4. [Online] https://www.handelsblatt.com/finanzen/maerkte/aktien/investieren-in-megatrends-wie-anleger-von-einer-fleischlosen-zukunft-profitieren-koennen/25032528.html?ticket=ST-575070-Yc7E2xfK2bd5rWodNjfV-ap6 .

5. [Online] https://cen.acs.org/business/food-ingredients/list-cleanmeat/96/i42.

6. Cultured meat from muscle stem cells: A review of challenges and. Isam T Kadim, Osman Mahgoub, Senan Baqir, Bernard Faye , Roger Purchas. 14(2): 222–233, s.l. : Science Direct, 2015.

7. [Online] https://www.forbes.com/sites/briankateman/2020/02/17/will-cultured-meat-soon-be-a-common-sight-in-supermarkets-across-the-globe/#17ebd06d7c66.

8. [Online] https://www.aocs.org/stay-informed/inform-magazine/featured-articles/clean-meat-february-2018?SSO=True#:~:text=Clean%20meat%E2%80%94also%20known%20as,food%20safety%2C%20and%20novel%20foods..

9. RethinkX. Rethinking Food and Agriculture 2020-2030 . 2019.

10. Coles CA, Wadeson J, Leyton CP, et al. . cattle., Proliferation rates of bovine primary muscle cells relate to liveweight and carcase weight in. doi:10.1371/journal.pone.0124468, s.l. : Plos One, 2015 Apr 15.