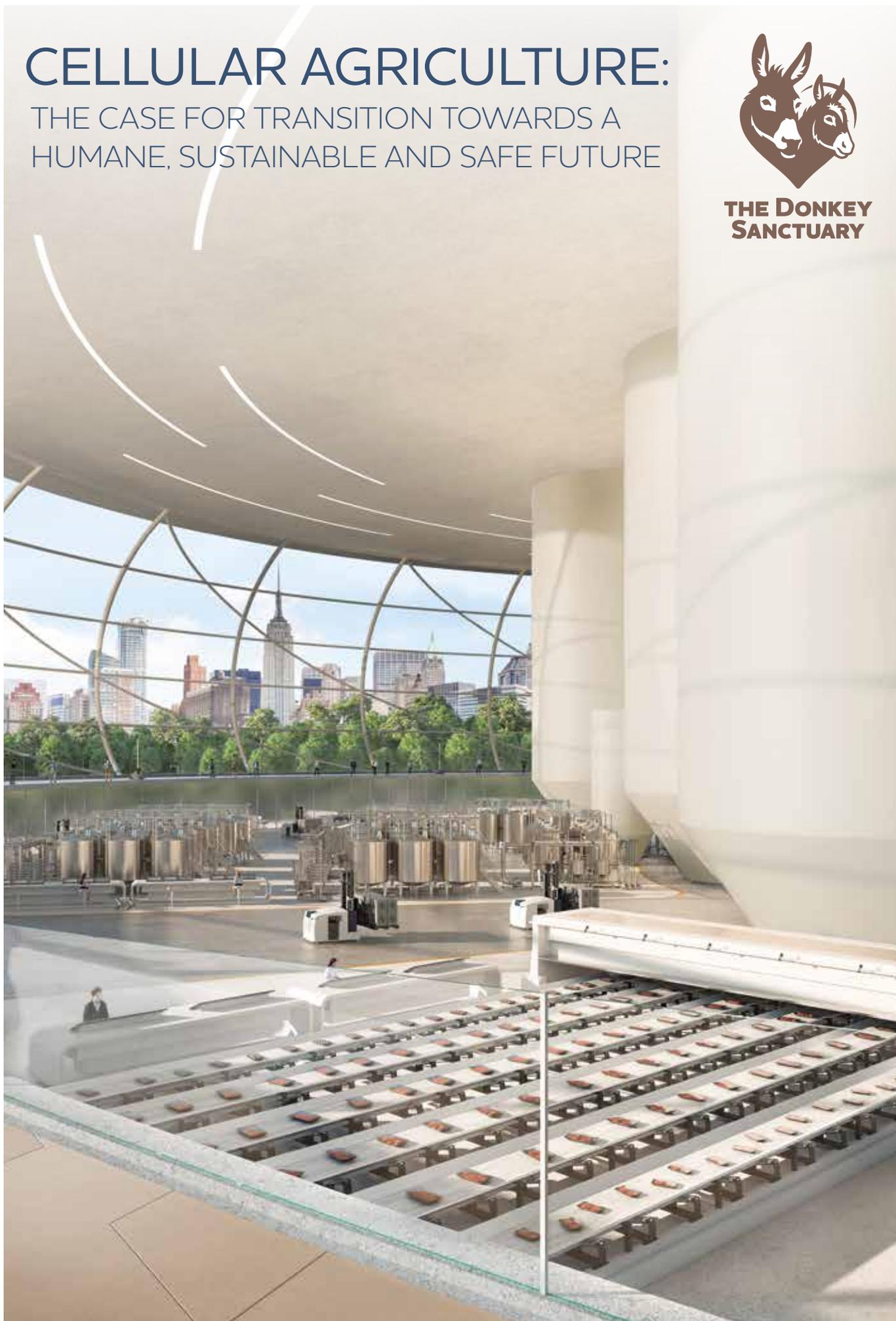


CELLULAR AGRICULTURE:

THE CASE FOR TRANSITION TOWARDS A
HUMANE, SUSTAINABLE AND SAFE FUTURE



**THE DONKEY
SANCTUARY**



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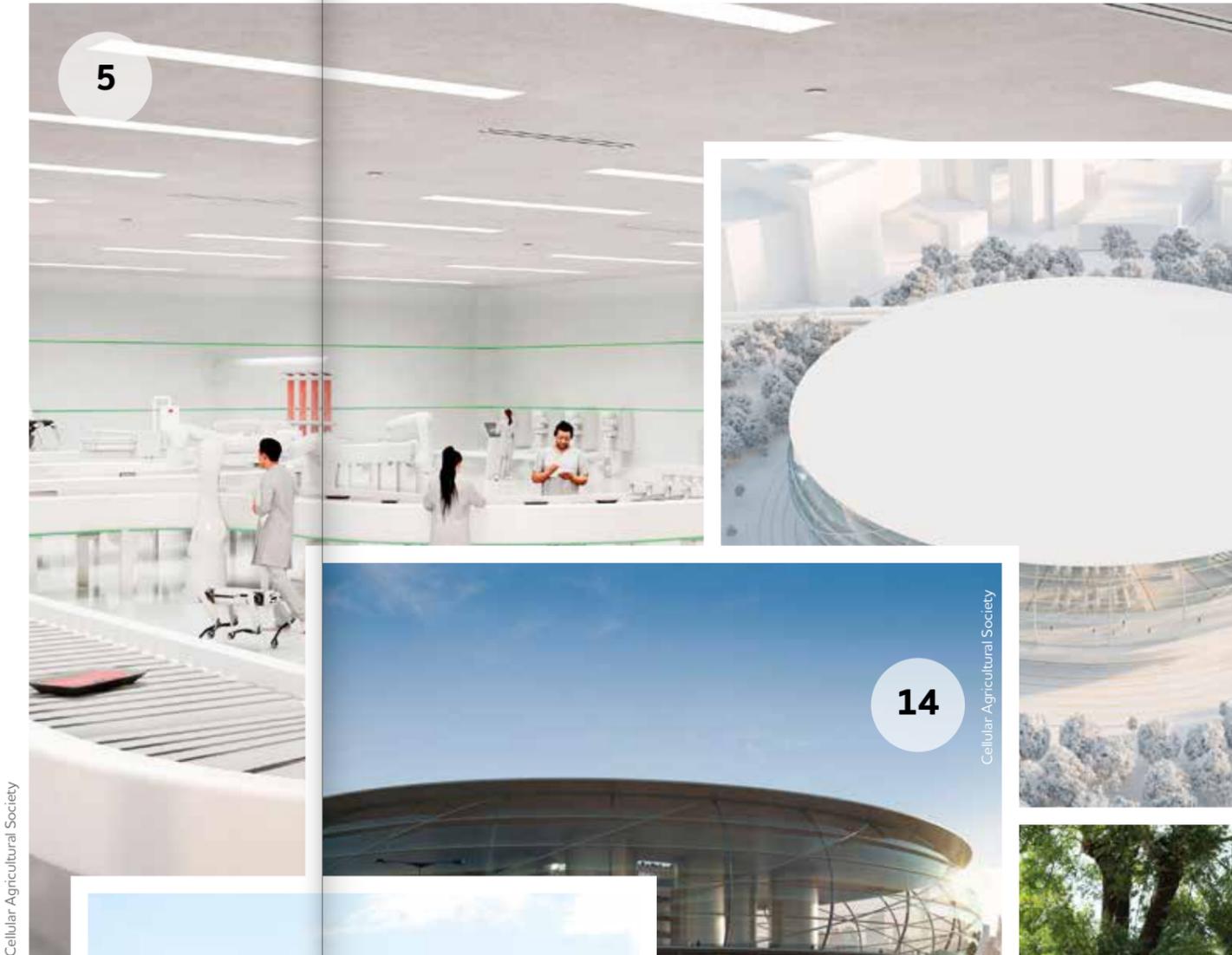
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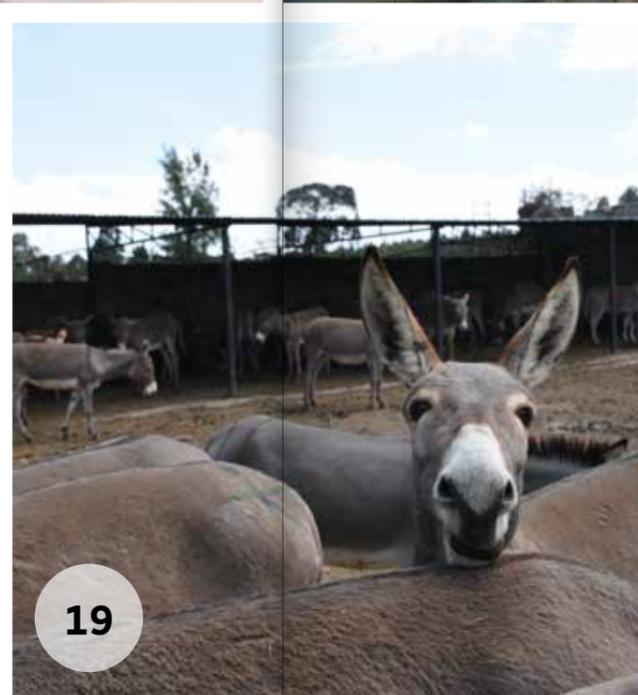
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EXECUTIVE SUMMARY

Ejiao is celebrated by many people as one of the treasures of Chinese culture and it has long been recognised as an important Traditional Chinese Medicine (TCM). Faced with increased consumer demand for ejiao products, and a dwindling domestic donkey herd, the ejiao industry has had no alternative other than to rely heavily on a global trade in donkey skins: a trade in which the movement of donkeys is largely unregulated, the disease status of donkeys unknown, and the slaughter and processing standards insufficient to prevent the spread of potentially harmful substances and diseases.

Chinese consumers are increasingly concerned with the safety of the products they consume and are willing to pay a premium for enhanced food safety measures and improved traceability. The current supply of raw materials for ejiao production leaves the industry highly vulnerable to concerns about product integrity

Donkeys are becoming increasingly difficult to source as their populations in some countries are depleted and a growing number of countries take steps to stop the trade in an effort to protect their national donkey herds, and the people who depend on those donkeys for their livelihoods.

Chinese philosophy has historically placed importance on treating animals well and, in recent years, a growing focus on animal welfare has been evidenced in Government initiatives, a growing domestic animal welfare sector, and in consumer expectations. This trajectory echoes the global trend towards greater compassion for animals: a trend that shows no sign of slowing in China or globally. Despite this, millions of donkeys suffer in the name of the global skin trade and are treated in ways that are unethical, unacceptable, and, quite often, illegal.

As the risks of the trade are better understood and more widely known, supply routes close, and donkey population numbers in some countries collapse, it is more important than ever that investment is made into humane, sustainable and safe solutions, where the demand for ejiao is met without compromising the welfare of donkeys, the survival of communities or the health of people or the environment.

Cellular agriculture offers the opportunity to combine the tradition of an ancient medicine

with innovative solutions for a sustainable future. It offers the promise of animal-free proteins that perform identically to their animal-derived counterparts across their entire functional and nutritional repertoire and a reliable, hygienic, safe supply chain that is unaffected by national bans on the export of donkey skins and increased scrutiny by the international community due to the animal welfare and biosecurity implications of the trade in donkeys and their skins.

While both precision fermentation and tissue cultivation could be employed to produce raw materials for ejiao production, precision fermentation is based on well-understood and widely used fermentation techniques and is likely to result in marketable products in a far shorter timeframe and require a far lower financial investment. As cellular agriculture technology is yet to be applied in the context of ejiao production, the costs and timeframes outlined in this report are speculative only and were estimated by extracting and adapting information related to the use of cellular agriculture in other fields. Thus, the methodology demands caution in the extrapolation of costs and timeframes to the specific production of donkey collagen.

That said, it is likely to take between one and two years to research and adapt precision fermentation technology for use with donkeys, and an initial investment of approximately USD163,000 to establish the production system required, excluding the cost of facility construction. Once established, it is initially likely to cost approximately USD5,200 to produce 1 kilogram of pure protein, excluding costs such as staff salaries, and this should be expected to decrease with time due to economies of scale. We estimate that, once production is established, it will take only six days to produce a batch of pure donkey protein using precision fermentation.

Tissue cultivation, on the other hand, is likely to take two to four years to adapt to this application and the fixed costs associated with establishing a system capable of cultivating donkey tissue are estimated to be approximately USD1,080,400 plus the cost of the salaries of research and development scientists, and any costs associated with constructing a facility. Once established, the variable costs associated with producing 1 kilogram of donkey tissue are likely to be

USD467,000 although this should be expected to decrease with time, again due to economies of scale. The time to produce each batch of donkey tissue is estimated to be approximately 26 days.

One kilogram of pure protein is sufficient to produce between 5 and 20kg of final ejiao product, using inclusion levels of 20% and 5% respectively, and both options provide an infinitely superior way to source raw materials than relying on a cruel, volatile, and unsustainable global trade in donkey skins.

A truly humane, sustainable, and safe solution is one that enables the ejiao industry to meet its need for raw materials without compromising donkey welfare, people's livelihoods, environmental protection, and human and animal health.

Now is the time to sever links with the global skin trade and to accelerate moves towards a humane, sustainable, and safe source of raw materials – the promise offered by cellular agriculture.



Cellular Agricultural Society



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INTRODUCTION

Ejjiao is recognised by many people as one of the treasures of Chinese culture¹ and is highly regarded by many consumers within China and globally. The ejiao industry, however, is heavily reliant on an international trade in donkey skins that is plagued with problems, including the biosecurity risks associated with the movement of large numbers of donkeys, opposition from a growing number of national governments, and negative impacts on donkey welfare, national donkey populations and on the people who rely on donkeys for their livelihoods.

These risks are ever increasing as donkey traders resort to progressively more desperate, and often illegal, measures to reach donkeys that are harder to obtain due to depleting numbers in many countries and a growing number of governments taking steps to end the trade. The future of the ejiao industry can no longer be reliant on fragile global donkey populations. It can no longer exploit donkeys that are a vital source of income for

many vulnerable people and that, in many cases, represent the difference between modest survival and destitution.

Recognising that the global skin trade is not sustainable, the industry has already taken steps to ensure skins can be sourced domestically but large-scale farming does not represent a viable solution to the rapidly dwindling supplies of donkey skins.

China has a long history of inventions that have shaped our world for the better² and this innovative spirit could be the key to transforming a supply chain from one marred by a multitude of issues to one that is truly humane, sustainable and safe. To find such solutions we may look to the field of cellular agriculture where groundbreaking advances in the production of animal collagen have set an encouraging precedent and may provide a promising option for the future of ejiao.



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THE NEED FOR A NEW SOLUTION

The global livestock industry has come under increasing scrutiny in recent years due to the scale of its environmental, ethical and human health impacts.^{3,4} Furthermore, the biosecurity risks associated with rearing, transporting, slaughtering and consuming animals, and animal products, have attracted increased global scrutiny following multiple disease outbreaks, not least the Covid-19 pandemic.

The global trade in donkey skins for ejiao production is no exception. Substantial evidence exists to demonstrate unequivocally that, due to the actions of some traders, the trade results in widespread donkey suffering, harm to the communities whose livelihoods depend on those donkeys, environmental damage and unmanaged

biosecurity risks that represent a danger to humans and animals alike.

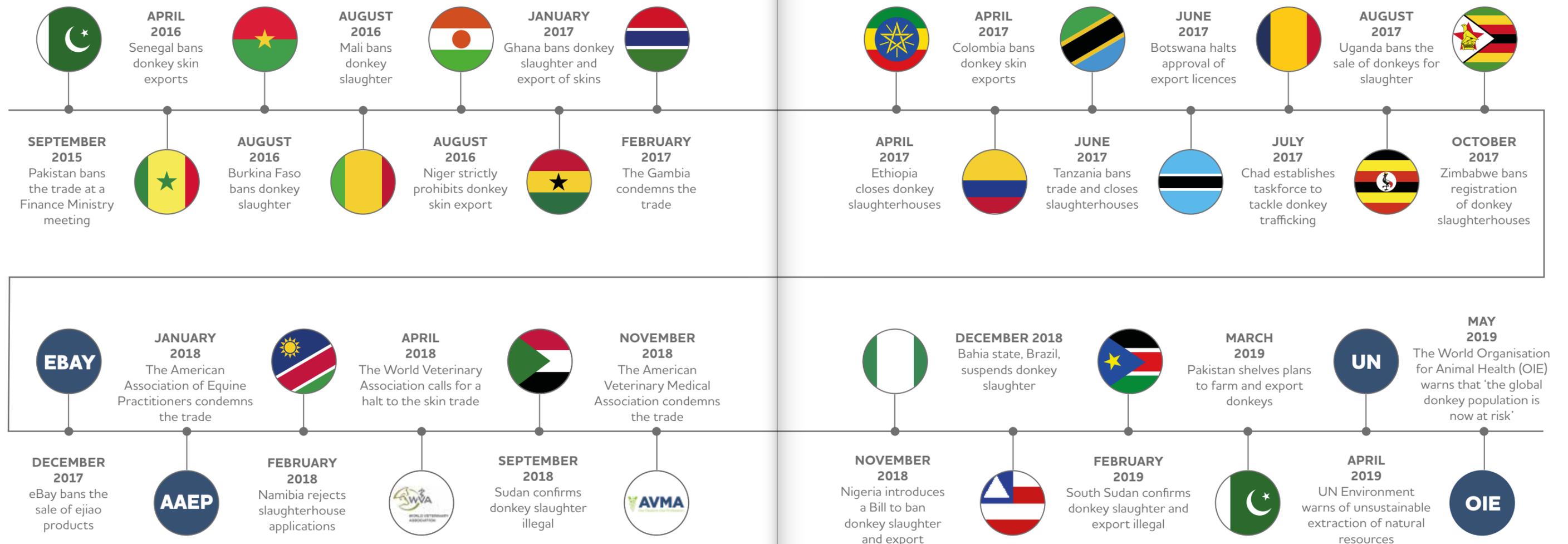
Global consumer trends demonstrate a growing concern for animal welfare,⁵ and an increasing expectation that animals used in production are treated humanely. These elevated concerns are partnered with greater awareness of the potential human health implications of the global livestock industry. High-profile coverage of livestock-related disease outbreaks such as Avian Influenza and African Swine Fever has led to increased public scrutiny of livestock industries.⁶

The Covid-19 pandemic serves as a timely reminder of the risks posed by unregulated trade and consumption of animals and their products.

The international trade in donkey skins for ejiao production is largely unregulated and creates a high risk of the spread of infectious diseases across the globe since the donkeys used are often of unknown health status and slaughtered in unhygienic conditions.

For these reasons, a growing number of national governments and international bodies are publicly expressing concerns about the impact of the international trade in donkey skins and are taking steps to end it.

As supply routes close, and donkey population numbers in some countries collapse, it is more important than ever that investment is made into humane, sustainable and safe solutions, where the demand for ejiao is met without compromising the welfare of donkeys, the survival of communities or the health of people or the environment.



DONKEY FARMING CHALLENGES AND POTENTIAL

As competition for the dwindling supply of donkeys intensifies, farming may appear to be an obvious solution by which a regular supply of skins can be secured. Due to the species' unique characteristics, however, farming does not represent a viable solution to supply shortages.

Donkeys' complex needs and long reproductive cycles make them unsuitable for many of the management practices associated with farming, particularly when it is done at scale. The farming of donkeys in any significant number requires a substantial investment of both time and money, and evidence suggests that it would likely take more than 20 years, and potentially far longer⁷ to reach the number of donkeys required to meet the industry's current demand for donkey skins. This prediction (figure 1) is based on ideal farming conditions, which may be difficult to achieve particularly as there is little

reliable information available on variables such as reproductive performance, mortality and breeding longevity for donkeys bred in large, intensive production systems.⁸

With limited research and reliable information available on the productivity of intensively farmed donkeys, the most favourable scenario makes assumptions based on knowledge of donkey reproduction and published research. Modelling (figure 2) uses an initial breeding herd of 200,000 donkeys with a breeding age of 24 months to 10, 12 and 15 years. It assumes a reproductive rate of 1.09, an average of 1.09 foals per jenny per year, and that 65% of foals will be female. This relies on a ratio of 1 jack to every 30 jennies for breeding purposes. Other assumptions are that slaughter will be at 17 months and mortality rates are 5% for foals and 2% for the adult herd.

Investment into donkey farming may address some of the shortfall caused by dwindling global donkey populations, but the high welfare standards required, and time constraints associated with growing the domestic donkey herd, make it an entirely inadequate option for addressing the immediate and growing supply crisis.

Even once established, there are, and will always be, inherent risks associated with donkey farming and transport. These risks include the impact of disease outbreak on the herd as well as the wider animal population, natural disasters, poor health and welfare outcomes, low productivity, low fertility and environmental degradation.

The risk of disease spread due to the movement of donkeys was demonstrated in West Africa during 2019. Reports of equine influenza in Nigeria were soon followed by unofficial reports of escalating mortalities along the recognised trade routes through the region. The spread of disease across borders and to communities of working equids along the route is an indication of the potential for devastation to the working equid population but also to the sports horse population.

A further example of this risk is the 2020 outbreak of African Horse Sickness, a highly infectious and deadly disease of equids, in Thailand. The disease spread from Africa to Asia, likely due to the importation of live animals, and infection spread rapidly across six provinces in Thailand before an equine movement lockdown was put in place. The outbreak led to a huge and avoidable risk being faced by all equines in Southeast Asia including high-value equines used in the lucrative horse racing and sports industries.

A truly humane, sustainable and safe solution is one that is free of these risks: one that ensures that the industry's need for raw materials is met without compromising the welfare of donkeys, or the health of people and the environment. While high-welfare farming of donkeys, potentially in showcase farms, could play an important role in the future of the industry, it will take decades for farming alone to supply sufficient raw materials. As such, farming does not represent a solution to the immediate challenges facing the ejiao industry.

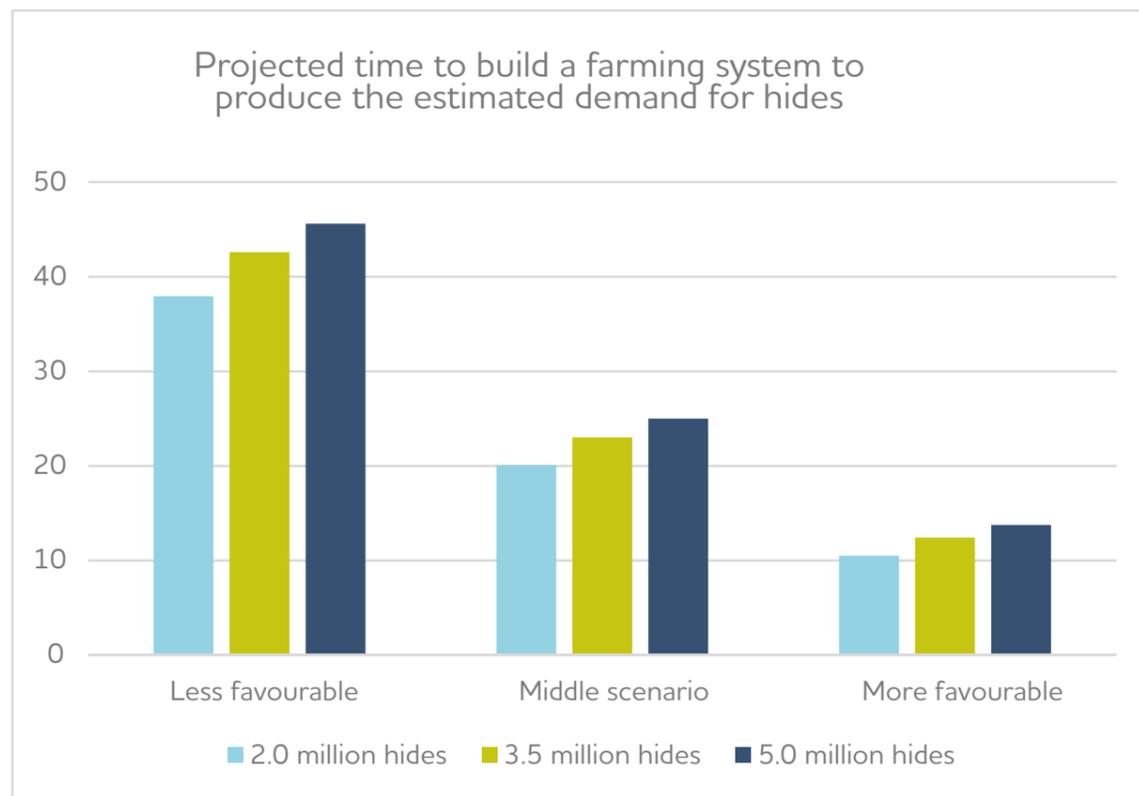


Figure 1. Number of years until the target hides of 2.0, 3.5 and 5.0 million per annum are reached under the three scenarios.

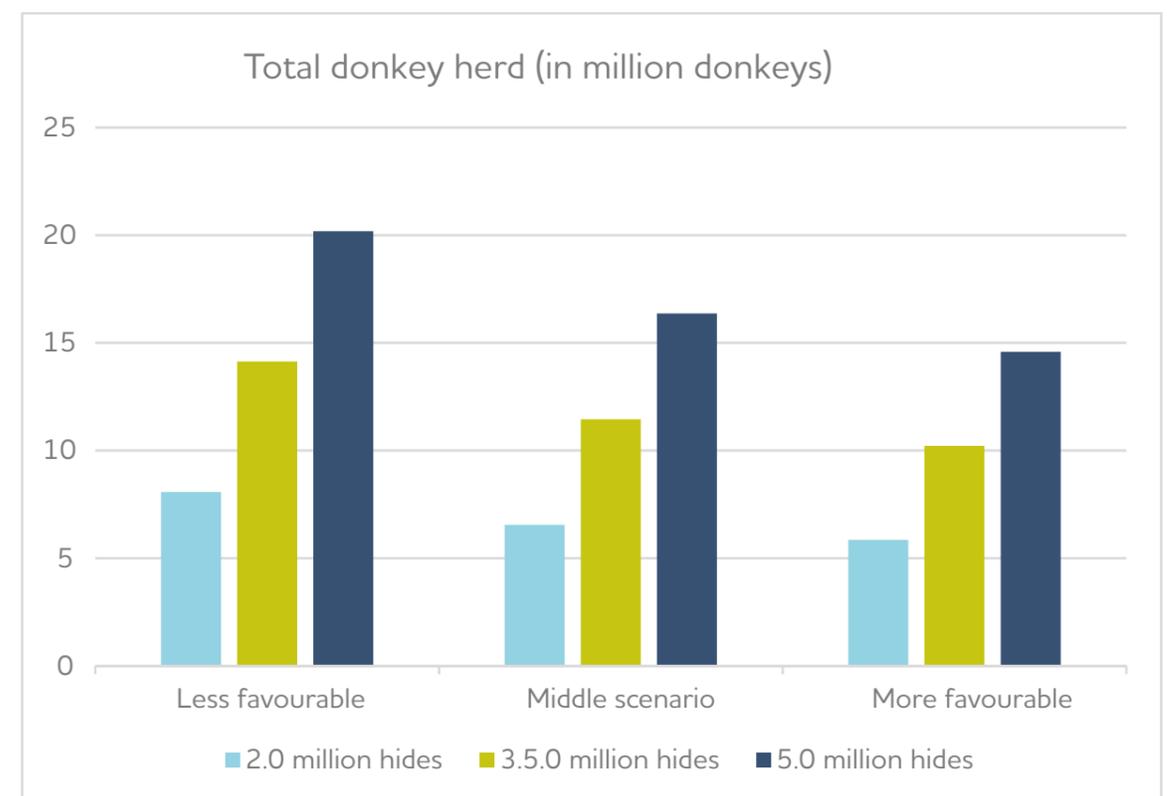


Figure 2. Total donkey herd required for the production of 2.0, 3.5 and 5.0 million hides per annum under the three scenarios.

Figures 1 & 2 reproduced, with permission, from Bennet & Pfunderer 2020, 'The potential for new donkey farming systems to supply the growing demand for hides', *Animals*, vol. 10, no. 4.

A TRANSITION TO CELLULAR AGRICULTURE

Cellular agriculture is the production of agricultural products, including animal products, using cell culture. Animal products produced using cellular agriculture do not require animals to be bred, reared and slaughtered⁹ but are biologically equivalent to the products made through traditional systems that do.¹⁰

The full potential of cellular agriculture is yet to be realised¹¹ but huge strides have been taken by the cellular agriculture industry in China and around the world. Groundbreaking advances in the production of animal collagen set an encouraging precedent and may provide a promising option for the future of ejiao production, with artificially grown, donkey-derived collagen grown in laboratories now a realistic prospect.

The rapidly growing field of cellular agriculture has arisen out of efforts to balance consumer demand for animal products with the need to improve animal welfare and reduce the environmental impact of animal industries.¹² Adopting a similar philosophy to the field of ejiao production, cellular agriculture may provide the innovative solution needed to ensure that consumers can continue to purchase ejiao but to do so in a way that protects donkeys, people and the environment.



Bioreactor with media culture by Sanofi Pasteur is licensed under CC BY-NC-ND 2.0



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Cellular agriculture encompasses a set of technologies to manufacture products typically obtained from livestock farming, using culturing techniques to manufacture the individual product.

Stephens, Di Silvo, Dunsford, Ellie, Glencross & Sexton 2018

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Cellular Agricultural Society



Cellular Agricultural Society

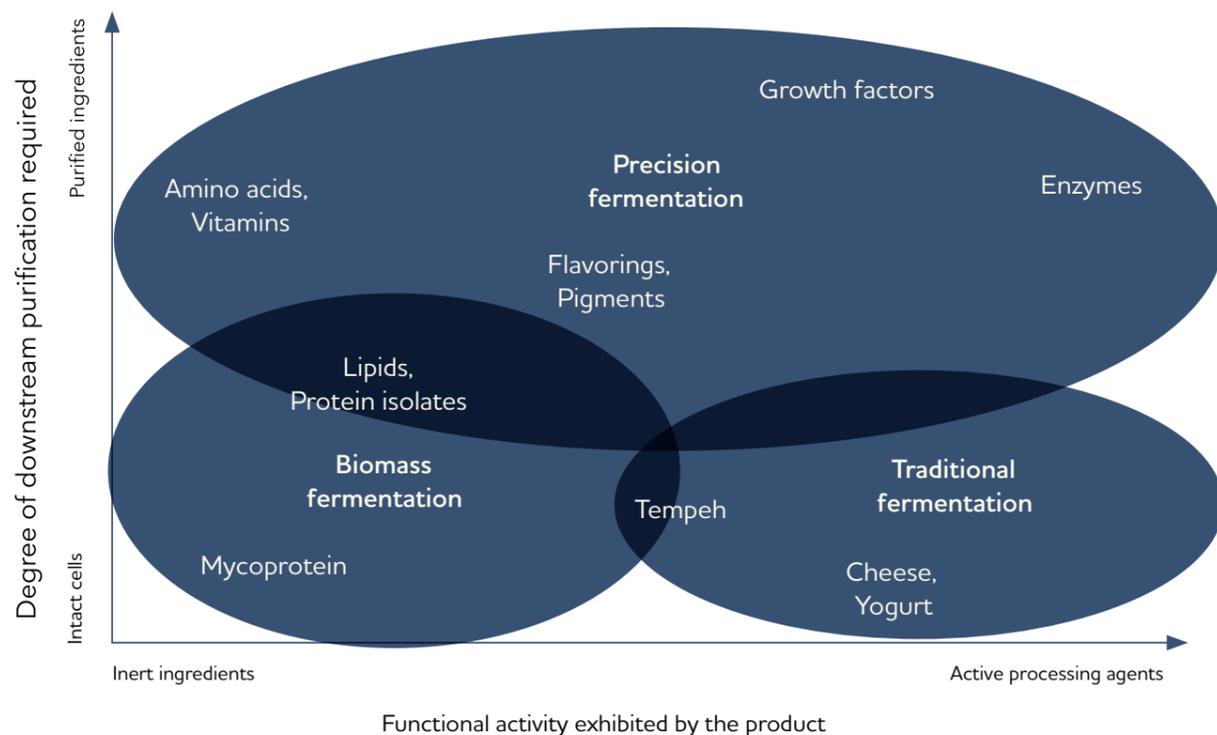
Production methods used in cellular agriculture can be divided into two broad categories: precision fermentation and tissue cultivation. While both methods appear promising for the creation of almost infinite supplies of biochemically identical collagen as a raw material, this result could be achieved with a far lower investment of time and money using precision fermentation.

PRECISION FERMENTATION – ‘USING MICROORGANISMS TO OBTAIN THE PROTEINS IN ANIMAL PRODUCTS’¹³

The technology for propagating and expressing recombinant genes was invented in 1973¹⁴ and offers the greatest potential for large-scale, cost-effective production of pure proteins.¹⁵ Recombinant collagen and gelatin are techniques

already widely used in human medicine and pharmacology applications¹⁶ and both are currently produced at an industrial scale.

Recombinant Deoxyribonucleic Acid (DNA) technology isolates a DNA sequence from one organism and introduces it into another organism, such as a yeast or bacteria, altering the genotype and phenotype of the recipient.¹⁷ The cells are then cultivated in fermentation tanks before the resulting protein, which is the same as the original animal-derived protein, is separated from the host cells. The novel technology, named *fermentation-based cellular agriculture*¹⁸ or *precision fermentation* is the combination of two processes (figure 3): precision biology or tailored biological production design and programming, and the well-known process of fermentation in a controlled environment.¹⁹



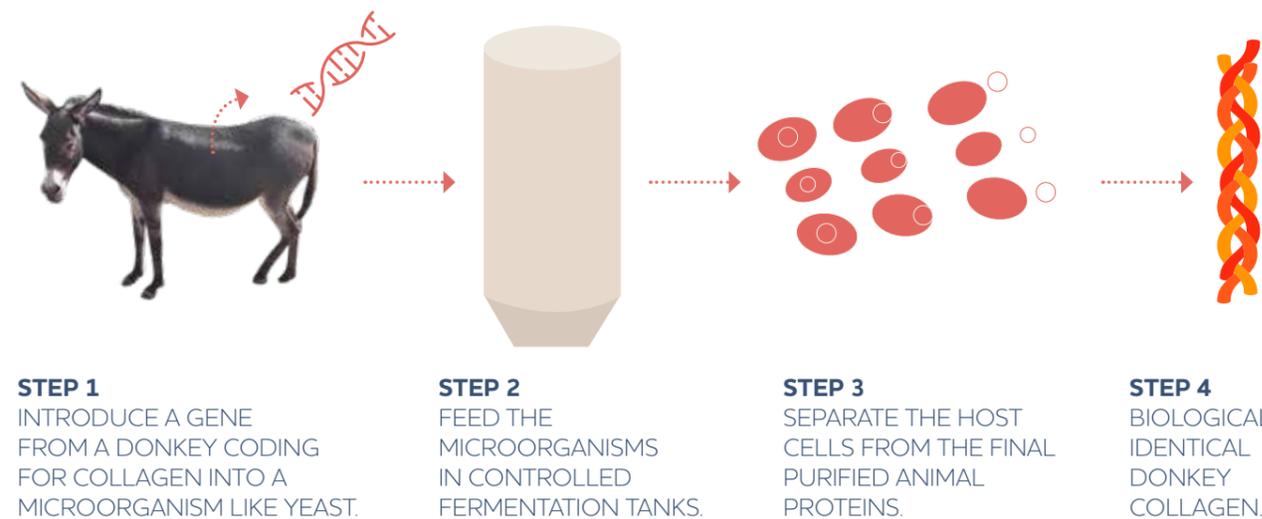
Source: GFI.

FIGURE 3. A conceptual landscape of fermentation-derived and fermentation-enabled products.

Reproduced, with permission, from the Good Food Institute's State of the Industry Report: Fermentation

The same process may be replicated for donkey skin gelatin production, using the appropriate species-specific collagen gene to create recombinant microorganisms and thus creating a cost-effective, scalable solution (figure 4).

As fermentation-based cellular agriculture draws on commonplace biotechnology, it may result in marketable products in a shorter timeframe than technology involving tissue engineering.²⁰



- STEP 1**
INTRODUCE A GENE FROM A DONKEY CODING FOR COLLAGEN INTO A MICROORGANISM LIKE YEAST.
- STEP 2**
FEED THE MICROORGANISMS IN CONTROLLED FERMENTATION TANKS.
- STEP 3**
SEPARATE THE HOST CELLS FROM THE FINAL PURIFIED ANIMAL PROTEINS.
- STEP 4**
BIOLOGICALLY IDENTICAL DONKEY COLLAGEN.

This technology can take several forms including:

- **Recombinant protein/collagen from bacteria (*Escherichia coli*) fermentation**
Bacterial hosts are commonly used for recombinant protein production and account for 30% of biopharmaceuticals on the market.^{21,22} The advantages of using *Escherichia coli* (*E. coli*) as the host organism are extensively documented: it has unparalleled fast growth kinetics; produces high density cultures; creates a rich complex media that can be made from readily available and inexpensive components; and transformation with exogenous DNA is fast and easy.²³ The generation, or doubling, time of *E. coli* is between 15–30 minutes²⁴ and a 21 fermentation using complex media will generate 50g to 80g (wet weight of cells), and assuming modest protein expression (2% to 5% of the total cellular protein), between 100 and 300mg of recombinant protein is available in the cells.²⁵
- **Recombinant protein/collagen from yeast (for example, *Pichia pastoris*) fermentation**
Yeasts are often used in synthetic biology due to their easy genetic manipulation and relatively low cost of maintenance.²⁶ Yeasts deliver higher viability of post-translational modifications such as protein processing, proper folding, disulphide bridge formation and protein secretion into the medium; growth under a wide range of pH, dissolved oxygen and temperature conditions.²⁷ Feeding strategies, including the addition of secondary carbon sources such as glycerol and sorbitol, have also been successfully used to increase the productivity of processes involving yeasts.²⁸

TISSUE CULTIVATION – ‘GROWING ANIMAL CELLS OUTSIDE OF ANIMALS’²⁹

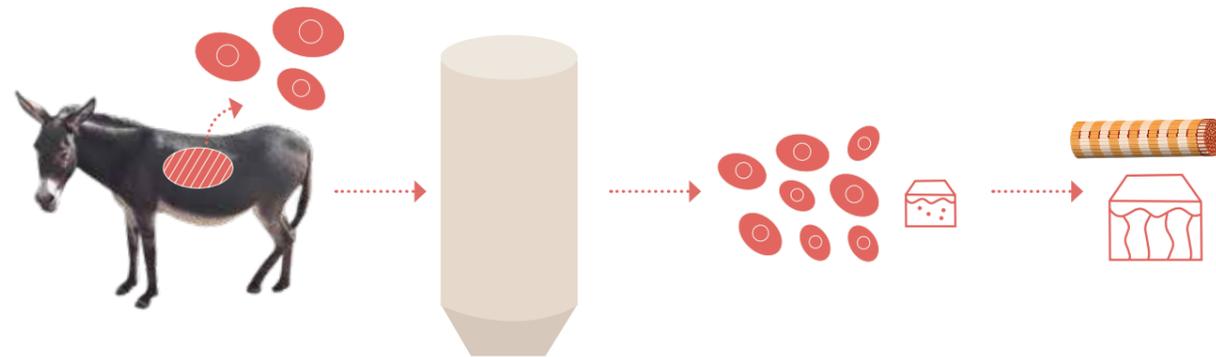
Tissue cultivation is a pioneering biomedical engineering discipline that integrates biology with engineering to create tissues or cellular products outside the body.³⁰ It has been used in human medicine to reproduce key structural and functional aspects of natural skin.³¹ In the context of an animal-related application, the ultimate goal is to produce authentic biological material,

grown in a laboratory environment, without the need for the original animal. Much of most advanced work in tissue cultivation is conducted by start-up companies that are naturally selective about how much information they share about their developments. As such, the extent to which detailed information can be obtained is somewhat limited. That said, the processes used in tissue cultivation have several critical steps.

A tissue sample is extracted via biopsy from an area of living skin under local anaesthesia and

satellite cells are isolated from the sample.³² Cells can be directly harvested from the target organ, developed from precursor or stem cells, or taken from lines grown in a lab. These satellite cells are stimulated via a culture medium to proliferate and differentiate through all phases of myogenesis, resulting in myofibres or small pieces of tissue.³³ Both these phases occur in bioreactors which provide complete control over conditions such as temperature, humidity and perfusion and the internal scaffold

or microcarriers to support the development of larger tissues.³⁴ The bioreactors in tissue cultivation perform a similar function to that of fermentation tanks in precision fermentation. After the multiplication phase, cells are deposited onto small sheets to induce collagen production. Finally, the tissues may be blended to form customized products.³⁵ Tissue cultivation would allow for similar processing stages to those used in current ejiao production methods.



STEP 1
TAKE A BIOPSY FROM A DONKEY AND ISOLATE THE SATELLITE CELLS.

STEP 2
FEED AND GROW THE SATELLITE CELLS IN CONTROLLED CELL CULTIVATORS CREATING MATURE TEMPLATE CELLS.

STEP 3
TRANSFER CELLS TO A SKIN CULTIVATOR WHERE THEY CHANGE FROM TEMPLATE CELLS TO SKIN CELLS.

STEP 4
UNDERTAKE A SCAFFOLDING PROCESS TO PROVIDE STRUCTURE TO THE FINAL MATERIAL, DONKEY TISSUE.

THE BENEFITS OF CELLULAR AGRICULTURE

Cellular agriculture offers the promise of proteins that perform identically to their animal-derived counterparts across their entire functional and nutritional repertoire.³⁶ This promise comes without the multitude of issues associated with the current global trade in donkey skins. Biological product sources that do not involve the rearing and slaughter of animals generate not only reduced environmental impact, but also provide enhanced product safety, increased efficiency³⁷ and traceability. Biological product sources also remove millions of animals from the process of production, thereby removing the risk of poor welfare, overcrowding, disease, and environmental impacts caused by livestock industries.

In the case of the global trade in donkey skins, a supply chain using cellular agriculture technology will also remove the unsustainable pressure currently placed on donkey populations globally, and the resulting impact on donkey-dependent communities.

Cellular agriculture can create a reliable, hygienic, safe supply chain that is unaffected by national

bans on the export of donkey skins and increased scrutiny by the international community due to the animal welfare and biosecurity implications of the trade in donkeys and their skins.

Cellular agriculture offers a wide range of benefits, below are those that are most relevant to the use of cellular agriculture to produce the raw materials required by the ejiao industry.

UNPARALLELED TRACEABILITY AND QUALITY CONTROL

Chinese consumers are increasingly concerned with the safety of the products they consume and are willing to pay a premium for enhanced food safety measures³⁸ and improved traceability.³⁹ An estimated 300 million Chinese are affected by foodborne diseases annually⁴⁰ and product contamination incidents occurring in China in recent years have heightened Chinese consumers' interest in the quality and safety of the products they consume. The current supply of raw materials for ejiao production leaves the industry highly vulnerable to concerns about product integrity.





The raw materials for ejiao production are, in part, sourced through a largely unregulated, sometimes illegal, international trade in donkey skins. As these skins are frequently derived from the unhygienic slaughter of donkeys of unknown origin and health status, traceability is virtually impossible. Many donkey traders are operating with minimal, if any, reference to the regulations that normally ensure the safety of health products globally. In many cases, unscrupulous dealers arrange the theft and cross-border trafficking of donkeys with no regard for the donkeys' health, or the risks associated with their movement and slaughter.

Donkeys are at risk of both contracting and spreading diseases during their often long journeys to slaughter. Donkeys are considered 'silent carriers' of diseases: they characteristically do not exhibit visible signs of ill health. They are also rarely subjected to ante-mortem or post-mortem inspection to determine health status. Hygiene during the slaughter of donkeys and the processing of their skins is poor, sometimes to the point of being non-existent. The preservation and storage of skins is unlikely to be completely effective in controlling harmful diseases and substances. It is also, in most cases, not compliant with food safety regulations.

The ease with which ejiao products may be contaminated by compounds dangerous to human health is alarming. The risk of

contamination is unsurprising when viewed in the context of the often unregulated, unhygienic and unethical way in which skins are sourced. Cellular agriculture offers unparalleled traceability and capacity for quality control. Cellular agriculture systems provide a fully traceable and controlled supply chain that dramatically reduces the biosecurity risks associated with sourcing raw materials for ejiao production. As it does not rely on the use of animals, the cellular agriculture supply chain will be both immune from, and not a potential contributor to, zoonotic and other disease outbreaks. Cellular agriculture products are completely traceable, they are guaranteed to be free of the biological pathogens and residues associated with live animals and they enable companies to eliminate the negative publicity and liability associated with unsafe or low-quality products.⁴¹ With adherence to stringent international standards on heavy metals and pesticide residue required for legal and legitimate access to international markets,⁴² including the European Union,⁴³ cellular agriculture could also offer the solution for overcoming these international trade barriers.

The capacity to establish hygienic and fully controlled systems that require minimal human interaction with products⁴⁴ enables industries to transcend the limits and challenges associated with traditional methods of producing animal-derived products. These limitations and risks could become a thing of the past.⁴⁵

THE PROTECTION OF DONKEYS

China has a cultural legacy of compassion for animals⁴⁶ and Chinese philosophy has, historically, placed importance on treating animals well. While China does not currently perform strongly in global animal welfare comparisons,⁴⁷ and there remains much room for improvement in the treatment of animals in the country, there have been areas of significant progress in terms of animal welfare over the past 15 years with both the Government of China and specific industries taking steps towards better animal welfare outcomes.

The establishment of the International Collaborative Committee for Animal Welfare (ICCAW) is one example of progress in the field of animal welfare in China. ICCAW's work has been instrumental in elevating the prominence of animal welfare in the country and its current efforts, alongside close to 1,000 companies, to develop farm animal welfare standards is likely to result in widespread improvements in the livestock sector. This commitment is further evidenced in joint initiatives between the Government of China and the United Nations Food and Agriculture Organisation (UNFAO) to promote awareness of farm animal welfare⁴⁸ and in the commitment made in 2017 by Vice Minister for Agriculture Yu Kangzhen to accelerate the introduction of comprehensive animal welfare legislation.⁴⁹

There is increasing consciousness of animal welfare issues within China⁵⁰ and attitudes are rapidly changing, particularly among young, educated, city-dwelling Chinese.⁵¹ Animal protection is considered by Chinese university students to be among the most important social progress movements in China, alongside

environmental protection and sustainable development.⁵² This is evidenced in the expansion of China's animal welfare movement and is a trajectory that echoes the global trend towards greater compassion for animals: a trend that shows no sign of slowing in China or globally.

This heightened awareness of animal welfare, in part fuelled by arrival of the internet information age and the rapid increase in the number of online communication channels and groups available,⁵³ is driving elevated expectations for the humane treatment of animals in food production, and animal welfare is now a component of food production that Chinese consumers are increasingly aware of and willing to pay for.⁵⁴

Donkeys are sentient and intelligent animals and, at the very least, they deserve a life that is free from suffering, and a death that is humane. The treatment of donkeys, and the intense suffering they can endure at every stage of the global skin trade, from sourcing to their eventual slaughter, is unethical, unacceptable and quite often illegal. The Donkey Sanctuary has an abundance of evidence of the abhorrent cruelty inflicted on donkeys in the name of the global skin trade, some of which is documented in its report *Under the Skin: An update on the global crisis for donkeys and the people who depend on them*.

A cellular agriculture solution would, unquestionably, eliminate the suffering of millions of donkeys in the name of ejiao production. It would also remove the global spotlight on the cruelty and suffering that is involved in the current trade. Once the initial cells required to start the production process are obtained, the requirement for donkeys, and the suffering this causes, is



entirely removed. Cellular agriculture promises the ejiao industry a completely humane solution.

SUSTAINABILITY OF SUPPLY

The current global trade in donkeys is entirely unsustainable. The unprecedented pressure placed on global populations has pushed some local populations to the brink of collapse, and has caused many national governments to take a stand against the trade in recognition of the critical role donkeys play in the survival of some of the world's most vulnerable communities.

With populations in some source countries collapsing, and an ever-increasing number of countries taking a stand against the trade, it is clear that the supply of skins through the international trade is finite, and that it is entirely inadequate for sustainably supplying the number of skins required each year for ejiao production. Cellular agriculture offers a sustainable and reliable source of raw materials. Following the sequencing of donkey collagen DNA and the establishment of manufacturing facilities and processes, an infinite supply of the raw material required for the manufacture of ejiao products could be produced as is already seen in the existing model of cellular agriculture meat production.⁵⁵ While the field of tissue engineering is still in a pioneering phase, the better-known process of precision fermentation could effectively decrease production time from the three years currently required to breed and rear a donkey to only a matter of weeks⁵⁶ as the

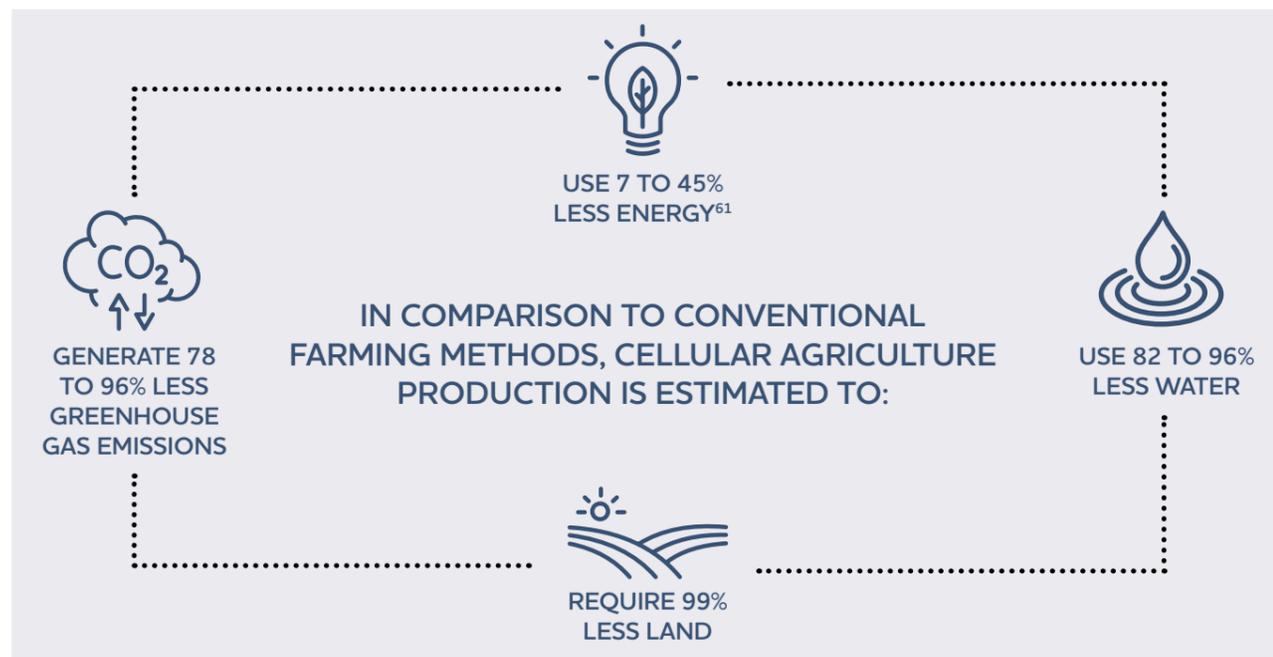
microorganism populations in this process double in hours, or even minutes.⁵⁷

ENVIRONMENTAL PROTECTION

Chinese consumers, like those in most countries, are increasingly conscious of the environmental implications of their consumption habits.⁵⁸ This awareness is an important market-driven factor generating growth in the number of environmentally friendly products available on the market, products for which some consumers are willing to pay a premium.^{59, 60}

The waste disposal practices associated with slaughter of donkeys for the global skin trade cause significant environmental degradation. As the skin is by far the most valuable part of any donkey slaughtered for the skin trade, there is often minimal incentive to use the remainder of the donkey carcass. These carcasses are either left in the open air or they are buried in makeshift pits where they pollute the nearby environment including, at times, the waterways that supply drinking water to nearby residents.

Cellular agriculture solutions have the potential to vastly decrease the environmental footprint of the ejiao industry. Not only would a cellular agriculture supply chain ameliorate the environmental damage caused by carcass disposal, but it would significantly reduce the land, energy and water required to produce the raw materials essential for ejiao production.



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A major advantage that favours the development of cell-based meat technology is that it has a lower environmental footprint compared to the conventional system.

Valente, Fiedler, Sucha Heidemann, Molento 2019

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MARKET POTENTIAL WITHIN CHINA

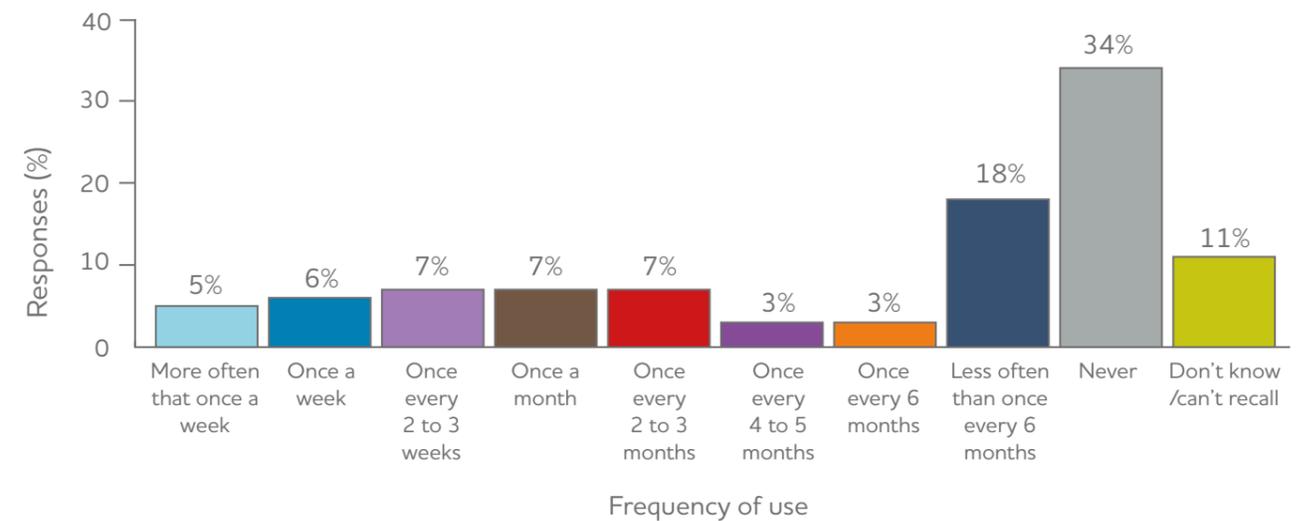
Consumer trends indicate that Chinese people are increasingly aware of the animal welfare, environmental and sustainability impacts of their choices and a growing number are willing to pay a premium for products that offer assurances on these points. Consumers are also more aware of the potential health risks associated with the products they consume. While cellular agriculture could offer an unparalleled opportunity to source the raw materials required for ejiao production in a humane, sustainable and safe way, it is also critical to understand how likely Chinese consumers are to try ejiao produced using this technology.

is significant interest amongst the Chinese population for ejiao produced using cellular agriculture. The fieldwork for this research was undertaken between the 6 and 10 of August and involved a nationally representative sample of 1,026 adults responding to an online survey. All figures are from YouGov and have been weighted to be representative of the Chinese population online.

This research shows that 11% of the population are currently frequent users or consumers of ejiao (once a week or more), 27% use or consume ejiao products occasionally, 18% rarely use or consume it and 34% do not use or consume ejiao at all.

Market research conducted by polling company YouGov in August 2020 indicates that there

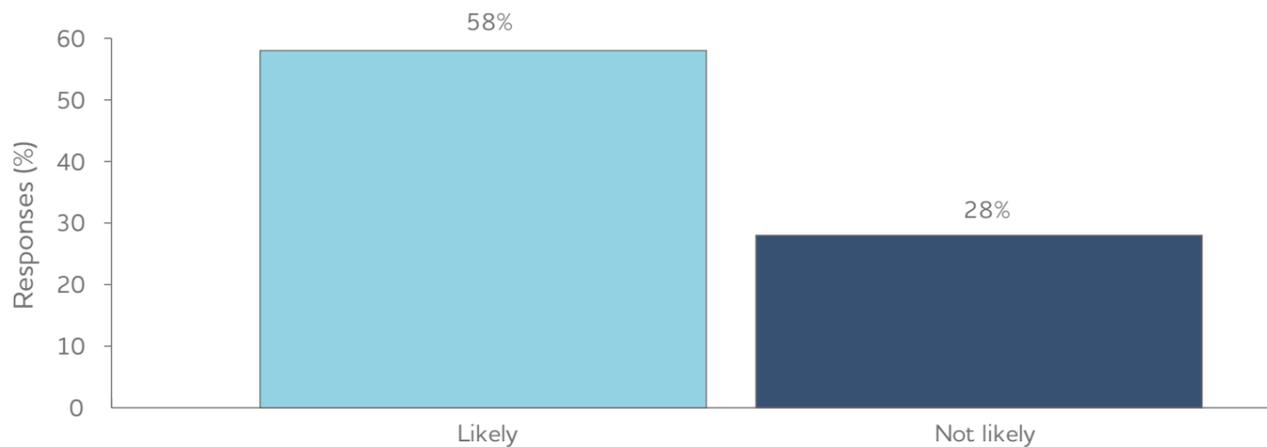
How often, if at all, do you think that you consume or use any products containing ejiao?



Encouragingly the results show that 58% of all respondents would be likely to use or consume ejiao products that were made using cellular agriculture if the products were available at an affordable price. The reasons given for this include a belief that the product may be more affordable (23%), an interest in trying new products (18%),

confidence in the use of advanced technology (8%), and because the product is likely to be good for their health (9%), equally or more effective than current products (8%), humane and environmentally sustainable (6%), better quality (5%) and safer (5%) than products currently available.

If available at an affordable price, how likely would you be to use or consume ejiao products produced by cellular agriculture?



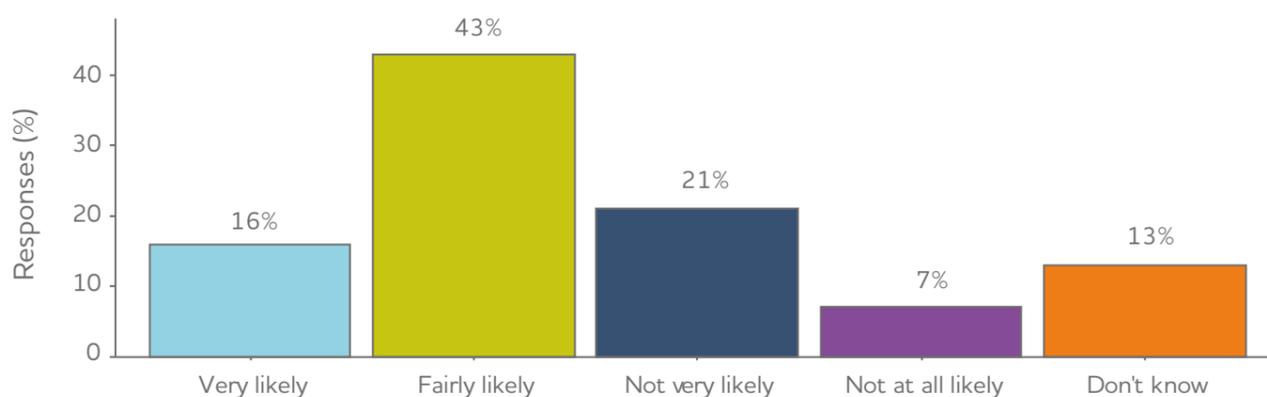
For those respondents who were unlikely to try ejiao produced using cellular agriculture, many expressed an aversion to, or disinterest in, ejiao as a product, rather than concerns about the use of cellular agriculture as a production method. 36% of respondents who were unlikely to try ejiao produced using cellular agriculture indicated that they either have no need for ejiao or no interest in consuming ejiao products, 5% indicated that they dislike the taste, and 4% said the products are too expensive.

indicated that they don't know much about (14%) or don't trust (11%) the process and that they thought it might not be as effective or good (10%) as current ejiao products.

Of the respondents who indicated they were willing to try ejiao produced using cellular agriculture, most (43%) indicated that they were *fairly likely* to try the products and a smaller percentage (16%) indicated they were *very likely*. On the reverse, 21% indicated they were *not very likely* and only 7% indicated that they were *not at all likely*.

Those respondents that specifically mentioned concerns with the use of cellular agriculture

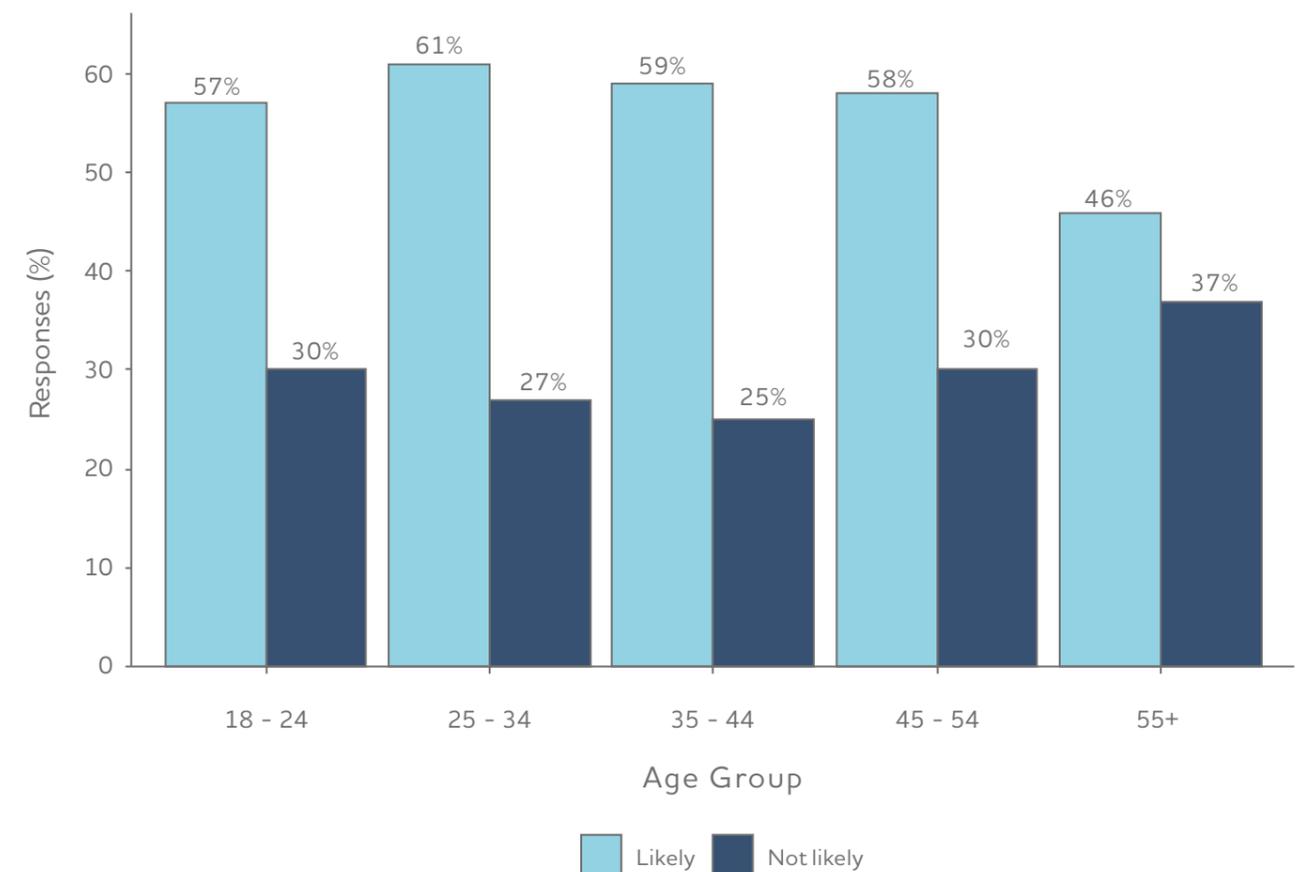
If available at an affordable price, how likely would you be to use or consume ejiao products produced by cellular agriculture?



These results are relatively consistent across all age groups from age 18 to 54 then the willingness to try ejiao produced using cellular agriculture drops slightly for people aged 55 and over. The high levels of receptivity among consumers aged 18 to 54 are encouraging for the future of the market as the young people of today have the potential to become lifelong ejiao consumers. They are also the consumers

whose purchasing is most influenced by animal welfare, environmental and sustainability considerations. Their willingness to use or consume cellular agriculture products, at a time when the field of cellular agriculture is still emerging, is highly promising and is likely to only increase once cellular agriculture is better understood and embraced by both Chinese and global audiences.

If available at an affordable price, how likely would you be to use or consume ejiao products produced by cellular agriculture?

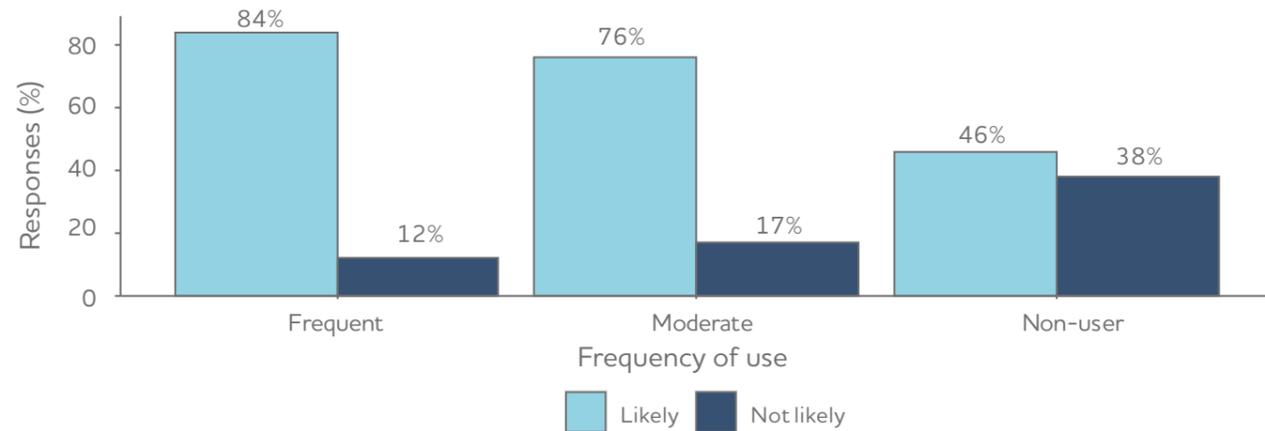


Frequent users of ejiao expressed a high degree of willingness to try cellular agriculture alternatives with 84% of frequent users responding that they were either *very likely* (29%) or *fairly likely* (55%) to try these alternatives if they were available at an affordable price. As these frequent users represent the most valuable market for the ejiao industry, this willingness is highly encouraging for the potential of cellular agriculture to be embraced as a solution to the current intractable issues facing the industry. Moderate users of ejiao were only slightly less likely to try ejiao products made using cellular agriculture with a total of 76% of frequent users

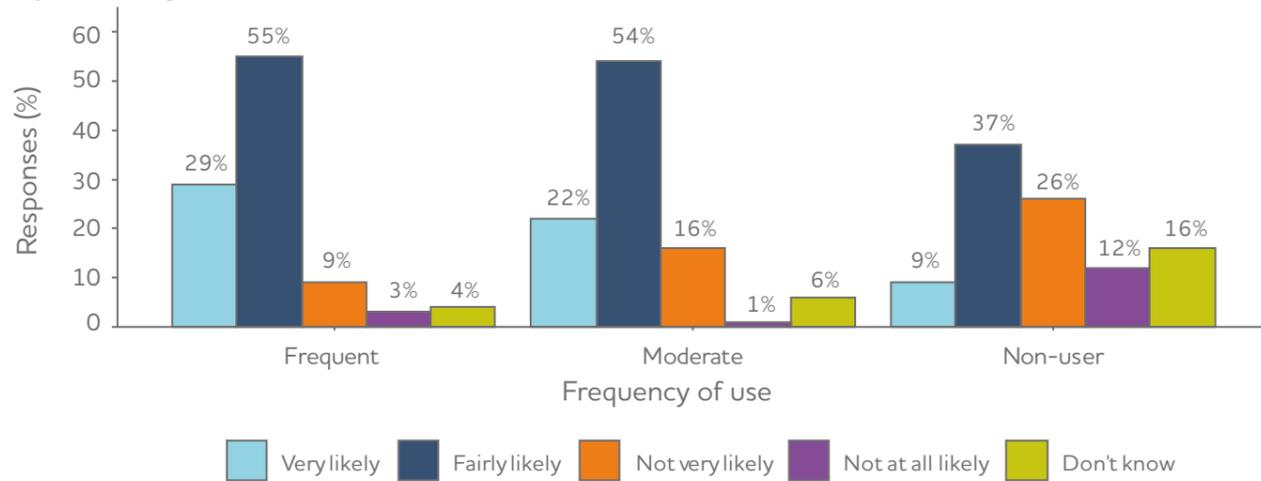
indicating they were *very likely* (22%) or *fairly likely* (54%) to try the products.

The survey results not only show the potential market within the cohort of existing ejiao consumers but indicate the possibility of reaching an entirely new market by transitioning to cellular agriculture. 46% of people who currently do not consume ejiao would be likely to use or consume ejiao produced using cellular agriculture, while 37% would be *fairly likely* and 9% would be *very likely*. This cohort represents an untapped market that cellular agriculture could provide the key to accessing.

If available at an affordable price, how likely would you be to use or consume ejiao products produced by cellular agriculture?



If available at an affordable price, how likely would you be to use or consume ejiao products produced by cellular agriculture?



BEYOND CHINA – A GLANCE AT THE EUROPEAN UNION

While the health maintenance role TCM plays for Asian communities is well recognised, it is becoming more frequently used outside of Asia⁶² but access to these markets relies on evidence of adherence to strict regulations governing traceability and product integrity.

Current production methods rely heavily on the global skin trade, a trade in which the movement of donkeys is largely unregulated, the disease status of donkeys unknown, and the slaughter and processing standards insufficient to prevent the spread of potentially harmful substances and diseases. There is often little, if any, possibility of establishing traceability and this represents a significant barrier to securing legal and legitimate commercial access to markets such as the European Union which, in the interest

of consumer safety, places strict controls on the production standards and traceability of food-related products.⁶³ The regulations that govern the import of composite products into the EU are poised to tighten in 2021 to prevent manufacturers claiming that products are exempt from control on the basis that the animal content of those products is below a certain percent.⁶⁴

The high degree of control and traceability possible in cellular agriculture is a world apart from the current unregulated, unhygienic and unsafe global trade in donkey skins. Cellular agriculture offers ejiao manufacturers the opportunity to exercise total control over their supply chains and in doing so, to provide the high levels of product safety assurance required by many countries.

TIME AND FINANCIAL INVESTMENT

The current global trade in donkey skins is cumbersome and it is costly. The costs associated with the trade are far more than just financial: animals, the people who depend on them, and the environment all pay a high price for this demonstrably unsustainable trade. The clean and controlled alternative offered by cellular agriculture solutions could not only significantly reduce the ongoing cost associated with sourcing raw products but would eliminate welfare and environmental implications, and the reputational risk associated with them.

As cellular agriculture technology is yet to be applied in the context of ejiao production, the costs and timeframes outlined here are speculative and indicative only and were estimated by extracting and adapting information related to the use of cellular agriculture in other fields. Thus, the methodology demands caution in the extrapolation of costs and timeframes to the specific production of donkey collagen, as particularities may arise and may impact both costs and timeframes in ways not possible to determine now.

The fixed costs outlined below, such as the establishment of equipment, are those related to the operation of a production facility regardless of production volume. The variable costs are defined as those that vary directly in relation to the workload of the laboratory, such as culture media.

PRECISION FERMENTATION

Of the two methods outlined, precision fermentation is likely to result in marketable products in a far shorter timeframe and at a much lower cost than tissue cultivation that relies on technology that is yet to be proven at scale.⁶⁵ Precision fermentation can be a slaughter-free process that provides unparalleled access to an almost infinite supply of pure protein. It will also improve the safety, traceability, reproducibility, quality and purity of the product. It is a well-known process and the technology required is readily available and extensively used in human medicine and pharmacological applications. It lends itself well to short-term implementation once the technology has been adapted for the purposes



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of producing donkey collagen and it is anticipated that the time required to research and adapt the technology for use with donkeys is between one and two years. The cost of precision fermentation has been steadily declining for the past 20 years from USD1,000,000 per kilogram in 2000 to only USD100 per kilogram in 2019. The cost is likely to continue to fall as more companies enter the market, and the technology becomes more efficient and adapted to a greater number of uses. It is anticipated that the cost of a pure protein product made using precision fermentation will be just USD10 per kilo in 2025.⁶⁶

The fixed costs associated with establishing a system capable of producing donkey collagen using



precision fermentation are outlined in Appendix A but are estimated to be just USD163,046. This figure does not include the costs that would be incurred by employing researchers to adapt precision fermentation technology for use with donkeys, nor costs such as the construction of laboratory and production facilities. The variable costs associated with producing 1kg of pure protein are currently estimated to be USD5,236, excluding regular laboratory costs such as facilities, staff salaries, and energy and water costs, (see Appendix B).

Therefore, excluding the initial investment required to establish the precision fermentation production systems, an investment that remains static and, as such, the costs associated with it are spread across the entire period during which the system is in operation, it is estimated that the cost of producing 1kg of pure protein using precision fermentation would be just USD5,236 plus the standard operating costs of such facilities such as staff salaries and operating expenses.

We estimate that it will take only six days to produce a batch of pure donkey protein using precision fermentation and it is likely that between 5 and 20kg of ejiao product could be produced from 1kg of pure protein, based on inclusion levels of 20% and 5% respectively. Once economies of scale are realised in precision fermentation, this technique would be significantly more time and cost effective than purchasing donkey skins from which only a very small amount of pure collagen can be derived.

TISSUE CULTIVATION

Tissue cultivation, by comparison, is far more embryonic in its development and, as such, is complex and currently more expensive. As tissue cultivation is a highly competitive and potentially very lucrative field, the start-up companies that are at the forefront of developing this technology are reluctant to share details of the process. As such, the specific timings and costings associated with cultivated tissue are difficult to determine.

That said, it is likely that the time required for research and development of the specificities of donkey tissue production, using tissue cultivation, will be approximately two to four years. In the absence of data specific to a donkey tissue application, this timeframe is based on data relating to fibroblast cultivation due to the potential similarity of these two techniques.

The fixed costs associated with establishing a system capable of cultivating donkey tissue are estimated to be approximately USD1,080,408 plus the cost of the salaries of research and development scientists, and any costs associated with constructing a facility, (see Appendix C). Once established, the variable costs associated with producing 1kg of donkey tissue are, at this time, likely to be USD466,890, (see Appendix D). Cost reductions are likely as economies of scale are realised.

Once production was established, we estimate that it will take only twenty-six days to produce each batch of donkey tissue using cultivation techniques.



CELLULAR AGRICULTURE IN PARALLEL INDUSTRIES

While ejiao products are unique and should not be entirely grouped together with other products for consumption, there are parallels that can be drawn from these examples and, together, they demonstrate the rapidly growing number of industries that are reaping the benefits offered by cellular agriculture technology. The application of cellular agriculture technology can be categorised into four key segments.

MEDICAL & HEALTH

Developments in cellular agriculture technology for medical purposes have been a significant catalyst for growth within the field of cellular agriculture and there continues to be great strides taken to develop and refine this technology for use in an ever-expanding range of applications.

Recombinant collagen and gelatin are techniques that already have widespread application in human medicine and pharmacology⁶⁷ and both are currently produced at an industrial scale. Biomedical applications including insulin,⁶⁸ wound dressings, graft coatings, suture material, drug release and tissue engineering.⁶⁹

At the forefront of innovation in the field of tissue engineering is the cultivation of human skin tissue

“Cellular agriculture offers an alternative production method that ameliorates all of the issues above while still delivering to the consumer a product that tastes and performs identically to the animal product it seeks to displace.”
Good Food Institute, 2018

to treat burn injuries. This work was, in part, pioneered by researchers at the First Affiliated Hospital of Sun Yat-sen University in Guangzhou alongside counterparts at Michigan Technological University. In 2016 this collaboration resulted in the successful use of an engineered sheet of stem cells in a skin graft on a rat.⁷⁰

Similarly, cellular therapy is also used in the equine industry, with companies such as the UK-based Equi-Stem, as well as multiple equine centres globally, leading research in the use of stem cell technology to treat equine injuries. Stem cell cultivation is also being undertaken by many smaller, general purpose labs making this technology increasingly accessible.

SKIN CARE & COSMETICS

Geltor, a biodesign company using fermentation to create high-value designer proteins, has recently released a range of topical products made using animal-free proteins. Using this technology Geltor can make not only familiar collagen proteins such as gelatin, but customised proteins with tailored nutritional and functional properties.⁷¹ Their range includes Collume™, a marine collagen, and HumaColl21™, a human collagen.⁷² Geltor has attracted investment of USD116.3 million and Geltor CEO has stated that 'our goal is to make it ridiculously easy for iconic brands to build sustainable products. The next stage of growth will allow Geltor to meet the moment our world is facing, as businesses recognise the urgent need to transition to a sustainable protein supply chain.'⁷³

In late 2019, Geltor signed a letter of intent with Gelita, a leading supplier of collagen proteins, which will see steps taken to develop, produce and market the first ingestible animal-free collagen protein.⁷⁴

FOOD

Cellular agriculture alternatives to animal food products is a fast-growing and diverse field which has seen a dramatic increase in both the number of companies established, and the level of investment made into the technology.

Precision fermentation has been used since the 1980s to create recombinant enzymes for cheese production⁷⁵ and recent developments in fermentation technologies, alongside the health-promoting benefits of fermented foods, are stimulating rapid growth and innovation in the fermented food sector.⁷⁶ There are currently 44 companies working with fermentation-based processes to obtain animal protein⁷⁷ and it is anticipated that this number will continue to grow in the coming 15 years.⁷⁸

Here are some examples of companies that use precision fermentation:

- Quorn, which uses fermentation of mycoprotein, a fungus-based culture, to make meat substitutes. An estimated five billion servings of Quorn have been consumed since products became available in 1983.⁷⁹
- Clara Foods is developing a range of products including animal-free egg proteins, nutritional supplements, baking products and food and beverage ingredients⁸⁰

- Perfect Day is producing the milk proteins whey and casein for use in the production of dairy products⁸¹
- Impossible Foods is producing plant-based heme which is made via fermentation of genetically engineered yeast.⁸²

CLOTHING

Both leather and silk have been replicated using cellular agriculture. Genetic engineering technology developed by Chinese scientists in the United States uses microalgae gene technology to produce expensive genetic technology biological products at low cost.⁸³ The first of these is spider silk, an extremely strong and tough material used in bulletproof vests, parachutes and airbags.⁸⁴ This animal-free spider silk is also used by companies, such as Bolt Threads, to produce a growing range of clothing.

In addition to its MICROSILK™ product range, Bolt Threads has developed a non-animal leather alternative using fermentation of mycelium cells found in mushrooms.⁸⁵ Modern Meadow is another company using recombinant technology to produce a non-animal leather alternative.⁸⁶



'Silk Stripes Scarf' by LollyKnit is licensed under CC-BY-NC 2.0



CONCLUSION

Faced with increased consumer demand and a dwindling domestic herd, the ejiao industry has had no alternative other than to source raw materials through the global skin trade. This highly vulnerable situation not only results in an unreliable supply chain but in catastrophic outcomes for donkeys and the people who depend on them, unacceptable biosecurity risks, and threats to both human and animal health.

There has been no viable alternative, until now. Cellular agriculture offers an innovative solution that could transform the ejiao supply chain. It is a solution that marries the tradition of an ancient medicine with innovative solutions that deliver biologically identical products, unparalleled traceability and control, the potential for a virtually unlimited supply of raw materials, and complete freedom from the multitude of negative impacts associated with the current trade.

Precision fermentation could provide the ejiao industry with unparalleled access to pure donkey protein in a relatively short timeframe, and with a modest investment. It represents the much-needed alternative to the global trade in donkey skin. It is a well understood and highly-scalable technology that, in just a handful of years, could be relied upon as a primary source of raw materials. If farming was still pursued for reasons of culture and tradition, it would no longer need to be relied on to supply skins and, as such, could be done so with a small number of high-welfare farms showcasing healthy Shandong donkeys, which also serve to preserve genetics useful for future development in cellular agriculture.

Cellular agriculture represents the only viable and safe way to produce the amount of donkey collagen currently required by the industry. Now is the time to sever links with the global skin trade and to accelerate moves towards a humane, sustainable and safe source of raw materials – the promise offered by cellular agriculture.

APPENDIX A

PRECISION FERMENTATION: FIXED COSTS

A preliminary estimate of fixed costs for the initial development of precision fermentation using yeast (*P. pastoris*). These costs are based on the initial research and development and many of these costs would not apply to ongoing production and, as such, industrial production costs would be far lower. Much of the equipment required for precision fermentation is readily available in existing laboratories.

ITEM	QUANTITY	COST USD
Autoclave	2	23,714
Balance	4	6,868
Bunsen burner	2	46
Bunsen burner hose	2	24
Centrifuge	2	28,922
Electroporator	2	5,448
Fermentation tank (700l)	4	36,436
Incubator shaker	2	5,998
Lab dryer	2	7,990
Refrigerator	2	15,714
Spectrophotometer	2	14,242
Thermal cycler	2	12,460
Water bath	2	5,184
TOTAL		163,046

APPENDIX B

PRECISION FERMENTATION: VARIABLE COSTS

A preliminary estimate of variable costs for the initial development of precision fermentation using yeast (*P. pastoris*). These costs are based on the initial research and development and many of these costs would not apply to ongoing production and, as such, industrial production costs would be far lower. Much of the equipment required for precision fermentation is readily available in existing laboratories.

ITEM	QUANTITY	COST USD
Agarose 1% 500g	1.6	580
Bam HI enzyme 2500u	1.6	80
Cdna synthesis kit	1.6	1,024
DNA loading buffer	1.6	38
DNTD mix	1.6	43
Electroporation cuvette 5u	1.6	24
Eppendorf tubes 500u	1.6	81
Geneticin	1.6	385
Gloves 100u	1.6	40
Goggles	1.6	20
Lab coats	1.6	25
Ligase buffer	1.6	56
Nucleospin kit	1.6	259
PCR buffer	1.6	195
PCR primers	1.6	297
PCR reaction mix 10u	1.6	19
Pipettes 12u	1.6	433
Primestar polymerase 1000u	1.6	1,329
Scraper 100u	1.6	51
Spei enzyme	1.6	139
Sterile water 1l	1.6	16
T4 DNA ligase 2000u	1.6	102
TOTAL		5,236

Note: this variable cost estimate does not include regular laboratory costs such as facilities, staff salaries, and energy and water costs.

APPENDIX C

TISSUE CULTIVATION: FIXED COSTS

A preliminary estimate of total fixed costs for the initial development of animal skin cultivation based on available literature and personal communication with specialists. These costs are based on the initial research and development phase and many of these costs would not apply to ongoing production and, as such, industrial production costs would be far lower. Much of the equipment required for precision fermentation is readily available in existing laboratories.

ITEM	QUANTITY	COST USD
3D bioprinter	1	200,000
Autoclave	2	2,328
Balance	5	11,640
Beaker	25	325
Bioreactor (50l)	1	609,680
Biosafety cabinet	2	2,328
Centrifuge	4	7,448
CO ₂ cylinder	4	1,864
Computer	3	9,076
Distiller	2	2,328
Drying oven	2	2,328
Erlenmeyer	50	1,500
Freezer	2	1,862
Hemocytometer	50	271
Hot plate stirrer	5	7,185
Incubator	5	22,340
Liquid N ₂ container	5	11,640
Magnetic agitator	2	466
Micropipette	20	4,660
Microscope	2	9,310
Peristaltic compressor pump	12	2,796
Peristaltic pump	12	19,548
pH meter	4	468
Pipette	200	4,800
Pipettor	8	14,896
Refrigerator	2	1,398
Test tube	100	1,200
Water bath	2	700
TOTAL		954,385

APPENDIX D

TISSUE CULTIVATION: VARIABLE COSTS

A preliminary estimate of total variable costs for the initial development of animal skin cultivation based on available literature and personal communication with specialists. These costs are based on the initial research and development phase and many of these costs would not apply to ongoing production and, as such, industrial production costs would be far lower. Much of the equipment required for precision fermentation is readily available in existing laboratories.

ITEM	QUANTITY	COST USD
15 to 50ml flasks	60	3,460
2ml flasks	100	7,620
Agarose 2% 25g 500ml	20	5,860
Agarose mode 10u	20	220
Alcohol	20	1,920
Antibiotics	40	1,040
Autoclavable glass flasks	200	5,960
Automatic pipet bulb	40	1,680
Beaker	2000	7,320
Brown paper	40	480
Capillary micropipettes 5000u	20	8,300
Cartridges 20u	20	4,900
Cell culture flask 250ml 75cm ² 5u	200	960
Cell culture flask 75cm 100u	20	5,120
Cell spreader	40	2,420
Coated dishes 100u	20	2,880
Copper sulphate 10g	20	1,320
Cryogenic tubes	20	2,220
Culture dishes 12 pits	2000	3,020
Culture dishes 24 pits	2000	5,740
Culture flask 25cm ²	60	15,360
Culture flask 50cm ²	60	15,360
Culture mediums	100	13,040
Disposable pipettes	40	4,400
DMEM low glucose	20	520
Dulbecco's PBS 1l	20	700
EDTA	20	8,380
Erlenmeyer 50, 100 and 500 ml	600	4,680
Ex-cell L	100	3,660
Foetal bovine serum	40	1,980
Filtration system to 500 ml	2000	3,120
Filtering unit	40	7,000
Gloves 100u	20	500
Goggles	20	260
Hepes 250ml	20	1,500
Lab coats	20	320
L-ascorbic acid	20	1,260
L-glycine 500g	20	9,140

APPENDIX D CONTINUED

ITEM	QUANTITY	COST USD
Low-cost modelling accessories to simulate a bioreactor (flasks, plastic accessories, filters, glass, samplers)	50	133,850
L-proline 100g	20	1,660
Microamp optical 48 well reaction plate	20	1,680
Microamp optical 96 well reaction plate	20	3,080
Micropipette tip 10u	100	5,640
Micropipette tip 200u	100	5,640
Micropipette tip 500u	100	5,640
Microplate 96 pits for thermocycler	1000	8,160
NaCl	60	4,500
Neubauer chamber	20	480
Paper towel	60	220
Parafilm	40	1,120
PBS salts	40	3,340
Penicillin Streptomycin	100	2,420
Pepsin for assay	40	15,020
Petri dishes	10000	2,340
Phosphate buffered saline 1l	20	800
Pipet bulb	60	500
Pipette tip	100	16,160
Porcine serum 10% 120mg	20	840
Porcine skin gelatin 500g	20	4,860
Roller bottles 850 cm ² 20u	20	4,840
Sanitizing	60	1,060
Scraper	2000	1,220
Serologic pipettes	60	1,720
Sterile filters	2000	3,080
Stripettor pipette accessories (U)	20	2,800
Syringe	1000	5,820
Test tube	2000	16,160
Trypsin	40	1,540
Tubes shelf	200	7,000
UltraPure LMP agarose 100g	20	10,440
Ultrapure water 100l	20	14,120
Vacuum filters	1000	15,520
TOTAL		466,890

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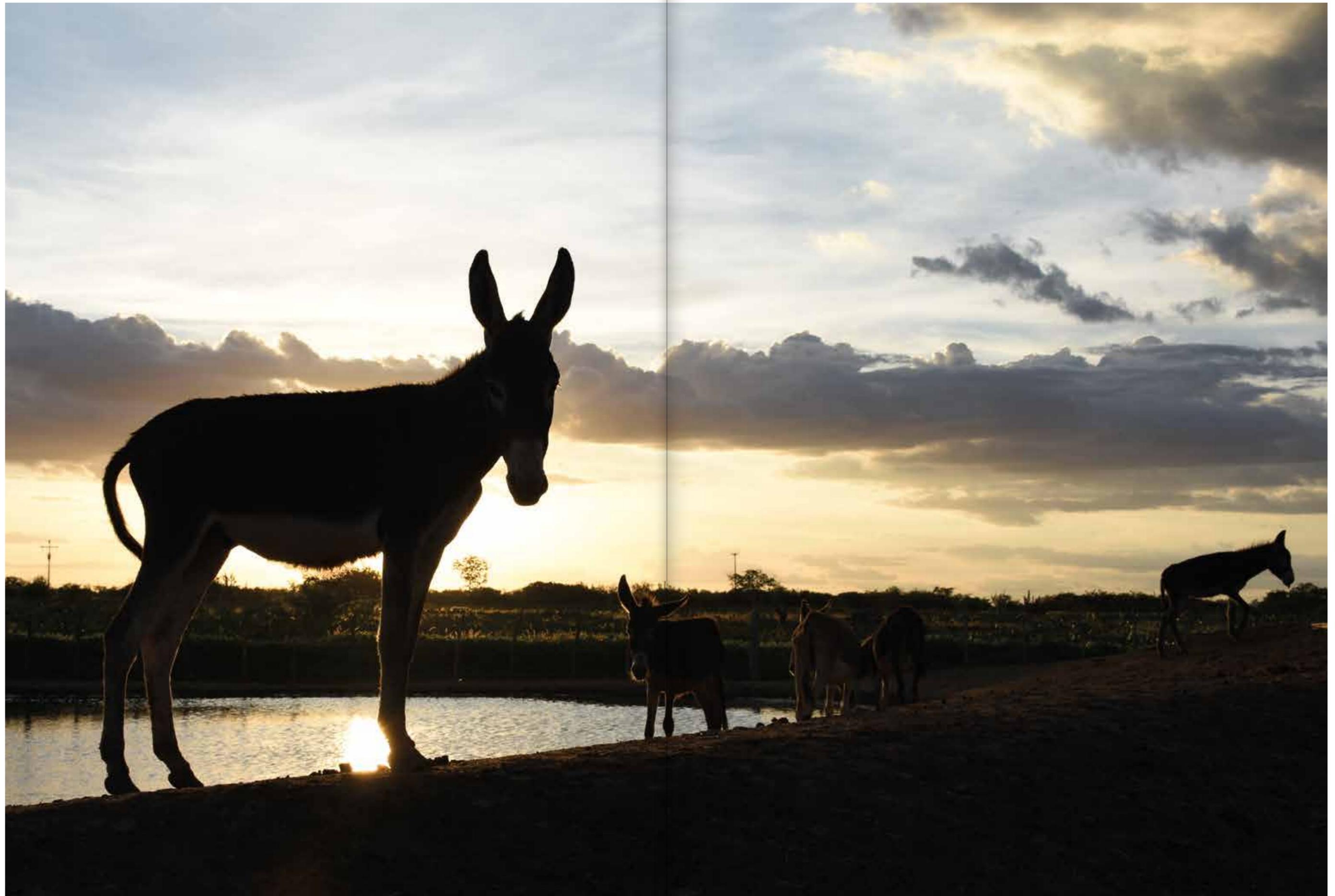
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The Donkey Sanctuary was founded by Dr Elisabeth Svendsen MBE in 1969. The Donkey Sanctuary (registered charity number 264818) and its sole corporate trustee, The Donkey Sanctuary Trustee Limited (Company number 07328588), both have their registered office at Slade House Farm, Sidmouth, EX10 0NU. Linked charities: The Elisabeth Svendsen Trust for Children and Donkeys (EST); The International Donkey Protection Trust (IDPT).