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Frontiers in Animal Science: Research and Development Volume II

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PREFACE

The dynamic field of animal science is at a pivotal moment in history, where the confluence of technological advancements, environmental challenges, and evolving societal needs is shaping the future of livestock production, animal health, and sustainable practices. "Frontiers in Animal Science: Research and Development" is a compendium of cutting-edge research, innovative practices, and forward-looking strategies that are redefining the landscape of animal science.

This book aims to provide a comprehensive overview of the latest developments in various sub-disciplines of animal science, including genetics, nutrition, physiology, behavior, and welfare. It seeks to bridge the gap between fundamental research and its practical applications, offering insights that are not only scientifically rigorous but also relevant to the real-world challenges faced by farmers, veterinarians, policymakers, and researchers.

Each chapter in this volume is authored by experts who are at the forefront of their respective fields, ensuring that the content is both authoritative and current. The topics covered range from the molecular mechanisms underlying animal growth and development to the application of precision agriculture technologies in livestock management. Additionally, the book addresses the ethical and environmental implications of modern animal production systems, emphasizing the importance of sustainable practices in ensuring food security and animal welfare.

As we look to the future, it is clear that animal science will continue to play a critical role in meeting the global demand for animal-derived products while minimizing the environmental footprint of animal agriculture. This book is intended to serve as a valuable resource for researchers, students, and professionals who are dedicated to advancing the field and contributing to the sustainable development of animal production systems.

We hope that "Frontiers in Animal Science: Research and Development" will inspire new ideas, foster collaboration, and drive the next wave of innovation in this vital field.

Editors

TABLE OF CONTENT

ADVANCES IN BYPRODUCT UTILIZATION IN SERICULTURE

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Abstract:

The pre-cocoon and post-cocoon processes in sericulture, which include raising silkworms and producing fabric, can all be grouped together. These activities typically result in a range of wastes. Therefore, by using the right techniques to recycle these waste products can now be converted into a useful enterprise that can increase farmers' revenue. Therefore, to ensure a profitable sericulture activity, these waste products from various sericulture activities must be processed to obtain biologically active substances with significant applications in various industries, including the food, pharmaceutical, and cosmetic sectors as well as in fisheries and livestock. Understanding the potential of using by-products produced from various sericulture operations, thorough study towards utility optimization is essential to putting the sericulture sector on solid ground. The review aims to highlight recent developments in the proper utilization, diversification, and value addition of sericulture resources in order to increase the industry's sustainability.

Keywords: Sericulture Waste, Utilization, Silkworm, Compost

Introduction:

Sericulture is an agriculture-based sector that primarily focuses on the production of natural raw silks. For farmers engaged in sericulture, the primary source of revenue is the production of silk cocoons. The production of food plants that silkworms can eat, which they then spin into silk cocoons, is the first step in the sericulture process. These cocoons are then rolled up and unwound to produce value-added products. Money moves from affluent to poor as higher-class consumers consume the final product users, adding sectoral value for rural households. Today, it is a significant cottage industry in a number of nations, including China, Brazil, North Korea, Thailand, India, Vietnam, and Uzbekistan. The top ten nations in the world that produce silk are shown in Table 1. Presently, nearly 95% of the world's total silk production is produced in China and India, the two largest silk producing nations. Because sericulture is a labour-intensive sector, it keeps nearly eight million people employed in rural India, securing their economics and preventing them from moving to larger cities (Manjunath *et al.*, 2020).

Table 1: Global silk production status (*Source: International Sericulture Commission, 2022*)

Global Rank	Country	Silk Production (Metric Tonnes)
1	China	50,000
2	India	36,582
3	Uzbekistan	2,037
4	Vietnam	1,236
5	Thailand	435
6	North Korea	370
7	Brazil	300
8	Iran	275
9	Bangladesh	41
10	Japan	10

Tasar, Muga, Eri, and Mulberry are the four main varieties of silk produced in India, giving it a special distinctiveness. Global production of silk is mostly attributed to the mulberry silkworm, *Bombyx mori* feed on mulberry plants. The natural way that Muga silk is produced is by raising Muga silkworms, *Antheraea assamensis* outside on host plants such as Som and Soalu trees. With five to six generations annually, these silkworms are essentially multivoltine, and they are now commercially raised in semi-domestic settings. In order to create Tasar silk, *Antheraea mylitta* rears its larvae on wild plants such as *Terminalia arjuna* and *Terminalia tomentosa*, while Samia ricini rears its Eri silk by feeding it castor leaves. Out of these four varieties, mulberry silkworms account for around 75% of the overall production of silk, with wild silks (also known as Vanya silks in India) sharing the remaining 25%. As a result, the word "silk" is typically used to refer to "Mulberry silk," whilst the other three are referred to as "Non-Mulberry silk" or "Vanya silk". In sericulture, the output of host plant cultivation serves as the input for the manufacture of cocoons, and host plant production is intricately linked to the production of cocoons. The constant pursuit of cocoon production in all silk kinds has resulted in the development of multiple technologies that are mutually dependent on sericulture and agriculture, which in turn produces a large number of wastes and secondary byproducts. People's perceptions of sericulture's sustainability can be significantly strengthened when it comes to the productive conversion of such waste materials into highly marketable goods. Working on strategies for the appropriate use of secondary wastes and by-products that can increase farmers' revenue is therefore essential. The following sections describe recent trends related to sericulture's use of byproducts.

West generation in sericulture practices

Pre-cocoon and post-cocoon activities are two broad categories into which all sericulture activities can be divided. The primary pre-cocoon tasks are the raising of silkworms and the cultivation of host plants up until the production of the cocoon. The primary silk wastes produced by these processes include dead larvae, moths, leaves, and silkworm litter. Reeling (silk extraction) and product development using different processing techniques, such as weaving, dying, finishing, etc., are included in the post-cocoon activities. Pupa, damaged or unusable cocoons (floss, multiple cocoons, perforated cocoons, stained cocoons, etc.) and fibrous silk waste are examples of potential waste products from these processes (Chaubey *et al.*, 2019; Kamili and Mosoodi, 2000). Fig. 1 illustrates the various waste types produced during the sericulture processes.

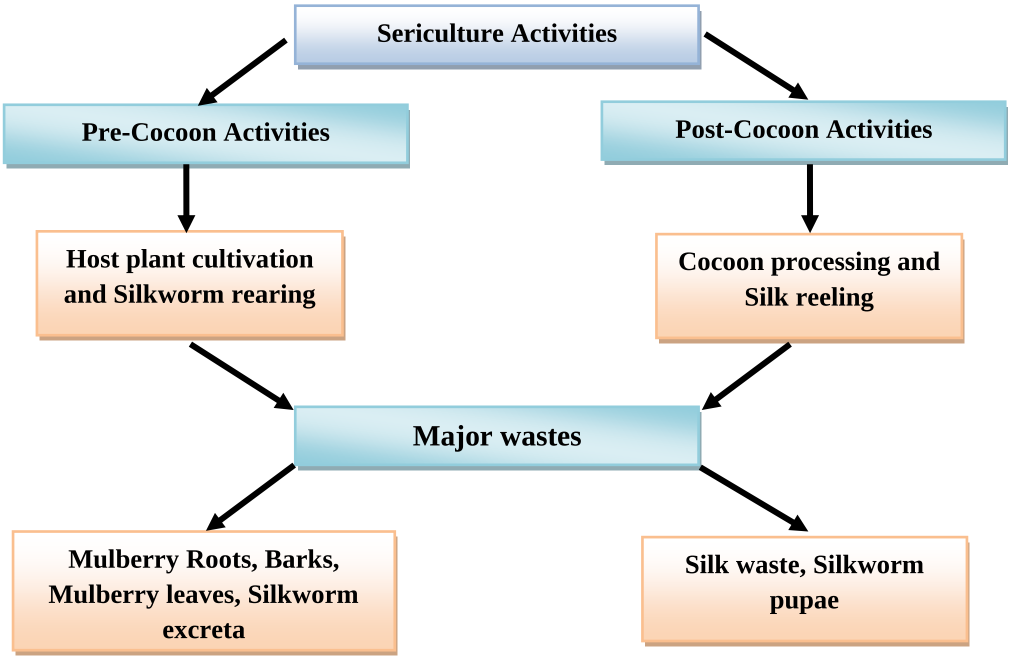

Fig. 1: Waste generation in Sericulture

Waste utilization from pre-cocoon sector

In addition to producing silk, farmers can make additional money through the appropriate use of sericulture's byproducts. Silk is a major textile fiber, and using by-products to make new products can help find non-textile applications where sericulture products work better than traditional materials.

1. Utilization as food and medicinal products

The mulberry plant (*Morus sp.*), which are the main food supply for *Bombyx mori* silkworms, have also been investigated for potential uses in other fields. Numerous studies have emphasised the mulberry's therapeutic qualities. Because mulberry fruit has high sugar content, it is used to make jellies, jams, and other sweet items. Mulberry fruit juice has been produced commercially as a health beverage in recent years, thanks to extensive work on cultivating mulberry plants under many conditions. It has gained a lot of popularity in China, Japan, and Korea. According to Dharmananda (2008), unadulterated mulberry fruit juice can be kept cold for three months, but when it is bottled, it can be kept at room temperature for up to a year. Carotene, glucose, sucrose, tartaric acid, succinic acid, vitamins B1, B2, and C, and sucrose are all abundant in mulberry fruit. In contemporary medicine, mulberries are only used to make syrup, which is then used to flavour and naturally colour medications (Singhal *et al.*, 2001, 2003). Due to their high anthocyanin content, mulberry fruits should be used to produce natural colour for use in culinary products. In the food sector, natural food colouring is in demand as synthetic pigments are dangerous. They are simple to extract and incorporate into aqueous food systems since they are soluble in water. There is already a low-cost, industrially viable way to extract anthocyanins from mulberry fruit (Liu *et al.*, 2004). This technology might be applied as a high-value food colourant or as a fabric tanning agent. The leaves of mulberries are used to make a decoction, which is essentially a herbal tea with blood sugar and cholesterol-lowering qualities. The leaves' extracts can be used to treat inflammation, irritation, and throat infections. They are also diaphoretic, meaning they promote sweating, and emollient, meaning they soothe. According to reports, the leaf extract can treat Alzheimer's by preventing the synthesis of amyloidal beta-peptide and lessening the neurotoxicity that results from this synthesis (Jain and Fillips, 1991). There have also been reports of improvements in tetanus and elephantiasis following leaf extracts treatment. It has been discovered that leaf juice is a useful treatment for amoebiasis, lung fever, endemic malaria, and diarrhoea (Grover *et al.*, 2002; Venkatesh Kumar and Chauhan, 2008). Mulberries are traditionally used as a feed in mixed forage diets for ruminants in many countries, including parts of Afghanistan, China, India, Bulgaria, Georgia, Azerbaijan, etc. Numerous studies on the use of mulberries for dairy cows and other domestic animals have been conducted in Italy (FAO, 1993). Similarly, a study effort was conducted in France to incorporate mulberries into livestock production (Armand and Meuret, 1995). Sheep and goats often like the high protein content of mulberry leaves (Takahashi, 1998). Mulberry leaves are an excellent addition to an Angora rabbit's diet for improved wool production, according to Singh *et al.* (Singh *et al.*, 1984). Moreover, mulberry introduction as animal feed has been attempted in nations such as France and Latin America (Armand and Meuret, 1995).

2. Utilization in compost preparation

Compost is nutrient-rich, aerobically regulated organic matter that has organically broken down. It can be used as a fertilizer, soil conditioner, and even a natural insecticide. Organic manures have a longer residual effect because the nutrients are retained in the soil for a long period and released more slowly (Bhatia *et al.*, 2013, Kumar *et al.*, 2013; Mukherjee *et al.*, 2018; Sharma and Mittra, 2007). Compost improves a plant's resistance to illnesses, pests, and other environmental stresses while also controlling erosion and reclaiming land and streams. Compost serves as a porous, absorbent substance that retains moisture and soluble minerals, giving plants the nourishment and support they need to flourish. It is combined with perlite, vermiculite, bark chips, sand, and soil. Composting material can be made from pre-cocoon waste, such as leftover leaves, excrement from larvae, and faeces. *Pleurotus florida* and *Pleurotus os treatus* are two fungi that can be utilised to break down this substrate and produce high-quality compost (Naik *et al.*, 1992). Anaerobic composting takes 120–150 days to finish, while vermicomposting takes 50–60 days (Kalaiyarasan *et al.,* 2015). Since the heat produced during the decomposition process might negatively influence seed germination, root growth, and the crop's capacity to quickly provide its actual nutritional demand, it is not advised to use the non-decomposed waste directly into the field. In addition, the likelihood of contracting an infectious disease can also be raised by the direct application of silk debris, particularly dead larvae. That's why it's critical to transform waste from sericulture into something good. Furthermore, research has revealed that the manure made from silk waste has superior nutrient qualities than farmyard manure (Kalaiyarasan *et al.*, 2015). So, compared to farmyard manure, compost made from sericulture waste has a greater potential to provide micronutrients (Sinha *et al.*, 2005). The amount of nitrogen in dried silkworm pupae is 8%. According to Mahesh *et al.*, (2020), there is a chance that pupal waste can be bioconverted to enriched compost and used as a source of nutrients because pupae include high levels of protein and nitrogen in addition to micronutrients like zinc, copper, magnesium, and manganese." Applying Silkworm pupae residual biocompost (SPRB) in conjunction with chemical fertilizers greatly improved the mulberry's development and yield characteristics (Mahesh *et al.*, 2020). Viable spore count (VSC) was used as a measure for assessing the effectiveness of pupal waste medium in Karthikeyan and Sivakumar's (2007) mass cultivation of the biopesticide bacterium *Bacillus thuringiensis* using silkworm pupal waste.

Vermicomposting sericulture waste was accomplished by introducing a mixed culture of juvenile earthworms, specifically *Eudrilus eugeniae*, *Eisellia felida*, and *Perionyx excavatus*, at a rate of 1.5 kwmt of wastes per trench (Singhal *et al.*, 2001). Addition of farmyard manure to sericulture waste can result in higher-quality vermicompost (Sinha *et al.,* 2005). Produce quality will also be enhanced by fortification using microbial inoculums such as Azotobacter, phosphate-solubilizing microorganisms, and single superphosphate (SSP) (Dandin *et al.,* 2000). In the form of silkworm rearing waste and other farm trash, one hectare of mulberry farm produces approximately 15 MT of sericultural waste annually. This amount is equal to 280–300 kg of nitrogen, 90–100 kg of phosphorus, and 150–200 kg of potash (Das *et al.,* 1997). When used as a vermicomposting starting material, these wastes provide organic manure that can significantly reduce the need for chemical fertilizers. It also improves soil health and mulberry plant nutrient accessibility, which improves leaf quality.

3. Utilization in biogas generation

The breakdown of organic matter during the anaerobic fermentation process yields biogas, a combination of various gases. Agro-ecosystems can benefit from the homogenous manure produced by the low-cost, environmentally benign, and sustainable process of producing biogas. Any type of waste material that can be successfully converted into biogas includes manure from farms, plant material, sewage, green trash, and food waste. Food leftover (leaf) and excreta from mulberry sericulture are combined to create the silkworm waste, which is a premium feedstock for the production of biogas. Biogas production has shown interest in using wastes such as defatted pupae of mulberry silkworms as a feedstock. Mao *et al.,* (2015) found that silkworm waste has an ideal C/N ratio of 15–35 and is composed of biodegradable organic materials. Another significant benefit is that it doesn't include any inhibiting substances, like detergents, antiseptics, or antibiotics (Dobre *et al.,* 2014). Because the inhibitory chemicals are poisonous to bacteria, they effectively impede the production of biogas through anaerobic fermentation. In addition to substrate quality, other variables that affect biogas production include an optimal process temperature, an effective hydraulic retention time (HRT), and an optimal organic loading rate (OLR) (Mao *et al.,* 2015). Due to the simple organic matter composition of silkworm waste and its excreta (Wang *et al.,* 2016), as well as the feedstock's low concentration of macromolecular compounds (Uzakova *et al.,* 1987), better methane yield (Kiran *et al.,* 2014; Niu *et al.,* 2013) are some of the reasons why it can ferment relatively quickly. Lochyn'ska and Frankowski (2018) discovered that silkworm breeding material has a higher potential for energy than silkworm excrement. According to Lochyn'ska and Frankowski's (2018) analysis of the composition and biogas yield of the substrates produced from mulberry sericulture, the calorific value of biogas generation was significantly raised by the high methane and dry matter contents of silkworm waste and excreta. The well-known biopesticide *Bacillus thuringiensis*, which is used to combat a variety of lepidopteron pests across the world, was successfully mass-cultivated using pupal waste as a medium by Karthikeyan and Shivakumar (2007) and Patil *et al.,* (2013). The anaerobic fermentation procedure used to produce biogas from silkworm excrement will not only increase agricultural manure's financial gain but also greatly enhance its homogeneity.

Waste utilization from post-cocoon sector

The post-cocoon sector deals with removing the raw silk from the cocoons and turning the fibrous silk into useful, valuable goods. "Reeling" is the process of removing (unwinding) silk filament from the cocoon; several cocoons are coiled together to create a single thread. Eri cocoons are the open-ended kind among the four varieties of silkworm cocoons; they cannot be reeled since continuous filament is not available for them. Hence, it is employed in the production of spun yarns (handspun or machine spun) that utilize all fiber material. Thus, very little waste is produced throughout the process of turning Eri cocoons into silk threads. Nevertheless, as the cocoons of Tasar, Mulberry, and Muga are made of continuous silk filaments, they are reeled in order to extract yarn made of continuous filaments. The two main by-products of the reeling process, which cannot be avoided, are "fibrous silk waste" and "pupal waste," aside from the creation of silk.

1. Utilization of pupal waste

Commercial silk manufacturing yields edible pupae as a byproduct, and an insect's economic value is increased if it can simultaneously generate two or more useful goods. Because of its special thermal qualities, *Samia ricini* produces Eri silk. The Brahmaputra valley and the areas inhabited by tribes were historically the main centers of Eri culture. Some adjacent state districts, including those in Manipur, Nagaland, Meghalaya, Mizoram, and Arunachal Pradesh, also practice it. On the other hand, its culture is spreading to other regions of India because of how simple it is to cultivate and how widely it is used. Long ingrained in the culture and traditions of Northeast India, Eri culture has a long history there. In Northeast India, Eri pupa is consumed by both regular Eri growers and tribes. The Ahom people eat Eri pupa at its mature stage, while other tribal populations in Northeast India take it at the pre-pupal stage. Rather than being raised for their silk, Eri silkworms are mostly raised for food in these regions. Some Asian countries also use the pupae of the mulberry silkworm as food (Sarmah, 2011).

Recent research indicates that silkworm pupae exhibit strong anti-tyrosinase activity and exceptional antioxidant capacity (Kwon *et al.*, 2012). The process of drying, deacidification, bleaching and molecular refines the pupal waste yields pupal oil. According to Harris *et al.*, (1997), these oil extracts are a good source of vitamin B2, which can be utilized to treat vitamin B2 insufficiency. Because of their ability to successfully cut triglycerides, they can be used to cure fatty liver, enhance blood quality, lower blood pressure, and stop arteriosclerosis. It can lower blood sugar by enhancing the actions of beta cells that produce insulin (Gavia *et al.,* 2003). The fatty acids in the oil have the potential to boost immune cell viability and increase fertility since they include natural steroids. Pupal oil contains α-linolenic acid, which has the ability to successfully prevent symptoms such as wrinkles, pigmentation, sallow skin, and premature ageing. The waste product of the silk industry and breeding operations, silkworm pupae, are rich in lipids and proteins and may be purchased for relatively little money. They make up 65–75% of the protein in cattle and poultry feed (Iyengar, 2002). The nutritional requirements of agricultural animals can be better met by using animal feed made from silkworm pupae, according to numerous researches. The hens' ability to lay eggs and the colour of their yolks are both enhanced by the de-oiled pupae meal. Hens' development rate and egg quality have been improved by the dried pupal meal. The fatless silkworm pupae produced higher yields when fed to fish (Buhroo *et al.*, 2018 a, b). Due of their high protein and fatty acid content, pupae were employed as food in dog, poultry, and piggery feed. Producing magur fish from silkworm pupae was also successful (Ghosh, 2005). Additionally, according to Buhroo *et al.*, (2018a, b), the dried pupal meal is effective in increasing fish survival, feed conversion, and specific growth rates. Because they were rich in fatty acids and protein, pupae were utilized as food in dog, poultry, and piggery feed. According to Ghosh (2005), it was discovered that the silkworm pupae were also incredibly economical to produce magur fish. According to Velayudhan *et al.,* (2008), the pupae's de-oiled diet improved the weight gain and fur growth of the rabbits.

2. Utilization of silk waste

Fibrous silk wastes are typically produced by the following processes: cutting and piercing cocoons from breeding and silkworm seed production centres; double cocoons, which are two cocoons spun together and cannot be reeled; extreme outer (floss) and inner (palades) layers of cocoons; waste leftover from reeling, winding, re-reeling, silk throwing, and other manufacturing processes. In comparison to reeled silk, "spun silk," a spun yarn produced from these fibrous wastes, is produced at a far lower cost. The fibrous silk waste materials undergo a combined processing of degumming and bleaching in order to eliminate sericin and colouring agents. To create thin fibre webs, the processed waste material lumps are opened, cleaned, and carded. A lot of these webs are consolidated and mechanically connected using the needle-punching technique, which involves a series of barbed needles going in and out of the web to align and interlock the fibres. Nonwoven fabrics of a given weight and dimension can be made in accordance with the specifications of the intended applications. These nonwovens have outstanding qualities that make them useful in many different applications. Some of these uses include geotextiles for soil reinforcement, filtration, the automotive and aerospace sectors, and medicinal applications (Manjunath *et al.,* 2020).

Fibroin and sericin, two important proteins, are components of silk. These proteins' distinctive architectures are characterised by sizable domains of hydrophobic amino acids divided by hydrophilic areas. Silk proteins are the preferred materials for tissue engineering and drug delivery because they are biocompatible, biodegradable, and have controlled shape. The waste silk can be efficiently used to extract fibroin and sericin, which have a variety of uses in pharmaceutical, cosmetic, and biomedical fields (Manjunath *et al.,* 2020).

Biocompatibility, biodegradability, and wettability are three amazing qualities of sericin that are used in the formulation of skin, nail, and hair cosmetic products (Padamwar & Pawar, 2003). According to Kitisin *et al.*, (2013), silk sericin has anti-aging properties that are similar to those of vitamin C, with the exception of oxidative stress, where it is superior because it can stimulate the synthesis of collagen type 1, suppress the regulation of nitrate, which may induce oxidative stress, and up-regulate the expression of b-cell lymphoma 2 (bcl-2), which inhibits cell apoptosis.

Summary:

In addition to producing cocoons, farmers can make additional money by properly using the waste and secondary by-products from sericulture. In general, silk wastes consist of fibrous wastes, agriculture wastes, pupal wastes, and silkworm wastes. Composting, biogas production, and animal feed are the main uses for agricultural wastes. While research on wild silk host plants has not yet been done, some mulberry herbs have been researched for their therapeutic qualities. Raising silkworm pupae for human food is the primary use of Eri culture in the North-Eastern states of India. Techniques for preserving pupae ought to be developed in light of the recent expansion of Eri culture throughout the rest of India. The Eri pupa is a waste product of sericulture because it is not regarded as food in the rest of India. Using the sericulture waste in an appropriate way will be aided by any processing and preservation technique that maintains the flavour and nutritional content of Eri pupa for at least two weeks. Developments are being made in the areas of vacuum packing, pickling, canning, dry snacks and so forth. If effective, this kind of exercise will prevent the wasting of tonnes of pre-pupa Eri silkworm in the remaining Indian states. Because sericulture has such a high potential for resource utilization, research institutions in advanced sericulture nations ought to focus more on this area of study in order to produce new, valuable products that make use of the abundance of silkworm germplasm and host plants that are available. By effectively utilizing the people capacity, research, and production facilities now available in the field of sericulture, the development of this field may be seen as a potential option to address the reduction in silk production.

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ADOPTION OF TECHNOLOGY IN INDIAN FISHERIES: BENEFITS AND BARRIERS

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Abstract:

The adoption of technology in Indian fisheries has the potential to revolutionize the industry by increasing productivity, sustainability, and profitability. Despite these benefits, there are significant barriers that hinder widespread technological adoption. This paper examines the benefits and barriers associated with the adoption of technology in Indian fisheries, highlighting key factors that influence the integration of modern technology in this sector.

Keywords: Technology Adoption, Indian Fisheries, Benefits, Barriers, Productivity, Sustainability

Introduction:

The Indian fisheries sector is an important part of the countrys economy generating jobs for millions of people and considerably contributing to food security fisheries and aquaculture have been identified as essential areas for supplying rising protein demand particularly in countries with a burgeoning population such as India traditional fishing tactics while historically effective confront several obstacles in fulfilling todays demands for sustainability and efficiency technological developments in fisheries can be critical in tackling these issues GPS navigation mobile market information applications automated feeding systems and satellite-based monitoring are changing fisheries management and operations these technologies have significant benefits including enhanced efficiency better resource management lower environmental impact and improved economic returns. However, the implementation of these technologies is not without obstacles. Financial limitations, lack of technical know-how, inadequate infrastructure, and resistance to change are significant barriers that need to be addressed. This paper aims to explore both the benefits and barriers of technology adoption in the Indian fisheries sector, providing insights into how these challenges can be overcome to harness the full potential of technological advancements.

Review of literature:

Chakraborty, S. (2022) examined the impact of digital technologies on small-scale fisheries in India, highlighting the improvements in data collection and market access. The study emphasized the importance of mobile applications in providing real-time information to fishermen. These applications have enabled fishermen to receive timely updates on weather conditions, fish prices, and fishing locations, thereby enhancing their decision-making capabilities and profitability. Rathod, P. (2022) explored the barriers to technology adoption in the aquaculture sector, identifying financial constraints, lack of training, and inadequate infrastructure as primary challenges. The research suggested policy interventions to facilitate technology uptake. Rathod pointed out that without sufficient financial support, many small-scale aquaculture farmers are unable to invest in advanced technologies. Additionally, the lack of access to training programs limits their ability to effectively use new technologies. Infrastructure deficiencies, such as poor internet connectivity and lack of modern equipment, further hinder the adoption process. The study recommended government subsidies and partnerships with private sectors to overcome these barriers. Sharma, V. (2023) conducted a study on the role of satellite technology in monitoring fish populations and improving sustainable fishing practices. The findings indicated a significant reduction in overfishing and bycatch with the use of satellite-based monitoring systems. Satellite technology allows for precise tracking of fish movements and environmental conditions, enabling more targeted and sustainable fishing practices. Sharma also highlighted the role of satellite data in enforcing fishing regulations and protecting endangered species. The study emphasized the need for increased investment in satellite technology and training programs to ensure widespread adoption. Moreover, the integration of satellite data with other technological tools can provide a comprehensive solution for sustainable fisheries management. Banerjee, A. (2023) analyzed the economic benefits of adopting automated feeding systems in fish farms. The study reported increased feed efficiency, reduced labour costs, and higher overall productivity as key advantages of technology adoption. Banerjee also noted that automated systems help in maintaining optimal feeding schedules, which improves fish health and growth rates, leading to better economic outcomes for fish farmers.

Objective of the paper

The objective of the paper is to investigate the benefits and barriers associated with the adoption of technology in the Indian fisheries sector. It aims to provide a comprehensive understanding of the factors influencing technological integration and offer recommendations for overcoming the challenges to maximize the benefits.

Technological advancements in indian fisheries

The integration of advanced technologies such as GPS, mobile applications, and automated feeding systems has significantly transformed the fisheries sector in India. These technologies have facilitated better navigation, efficient resource management, and enhanced communication among stakeholders. Mobile apps provide real-time weather updates, market prices, and catch data, enabling fishermen to make informed decisions. Automated feeding systems and sensors help in optimizing feed usage and monitoring water quality, thereby improving fish health and yield. GPS technology has revolutionized navigation and tracking for fishermen, allowing them to locate fish schools more accurately and reduce fuel consumption. This precision not only saves time and resources but also minimizes the environmental impact of fishing activities. Mobile applications have become indispensable tools, offering features like digital logbooks, catch recording, and direct access to marketplaces, thus eliminating middlemen and ensuring fair prices for their catch. These apps also facilitate peer-to-peer knowledge sharing and community building among fishermen. Automated feeding systems in aquaculture ensure that fish receive the right amount of feed at the right times, reducing wastage and improving growth rates. Sensors and Internet of Things (IoT) devices monitor water quality parameters such as temperature, pH, and oxygen levels, providing real-time data that helps maintain optimal conditions for fish health. Furthermore, blockchain technology is being explored for traceability and transparency in the supply chain, ensuring that consumers receive high-quality, sustainably sourced seafood. These technological advancements collectively enhance the efficiency, profitability, and sustainability of the fisheries sector in India.

Socio-economic impact of technology adoption

The adoption of technology in fisheries has profound socio-economic implications. Enhanced productivity and efficiency lead to higher incomes for fishermen and reduced operational costs. Improved sustainability practices contribute to the long-term viability of fisheries, ensuring a stable source of livelihood for future generations. Technology also empowers women and marginalized communities by providing them with access to information and market opportunities, thereby promoting inclusive growth in the sector. The increase in income for fishermen due to higher yields and better market access translates into improved living standards and economic stability for their families. The reduction in operational costs through efficient resource management and reduced wastage allows for reinvestment into the fisheries, fostering a cycle of continuous improvement and growth. Sustainable fishing practices, supported by technology, help preserve marine ecosystems, ensuring that fish populations remain healthy and abundant for future generations. Moreover, technology adoption facilitates better education and training opportunities for fishermen and their families, enhancing their skills and knowledge. Women, often involved in post-harvest activities, gain access to digital tools that improve their efficiency and market reach, thereby increasing their economic contribution. The inclusion of marginalized communities in the technological advancement of fisheries promotes social equity and reduces poverty. Overall, technology adoption in fisheries not only drives economic benefits but also fosters social development and environmental stewardship.

Statistical data on technology adoption in Indian fisheries (2022-2023)

Indicator	Value	Source
Total fish production (2021-22)	162.48 lakh tonnes	Handbook on Fisheries Statistics 2022 (People's Archive of Rural India)
Inland fish production (2021-22)	121.21 lakh tonnes	Handbook on Fisheries Statistics 2022 (People's Archive of Rural India)
Marine fish production (2021-22)	41.27 lakh tonnes	Handbook on Fisheries Statistics 2022 (People's Archive of Rural India)
India's rank in global fish production	3rd	Department of Fisheries, GoI (Department of Fisheries)
Total fisherfolk population	28.06 million	Handbook on Fisheries Statistics 2022 (People's Archive of Rural India)
Female fisherfolk population	12 million	Handbook on Fisheries Statistics 2022 (People's Archive of Rural India)
Fish products export value (2021-22)	Rs. 57,586 crore	Handbook on Fisheries Statistics 2022 (People's Archive of Rural India)
Fish products export quantity (2021-22)	13.6 lakh tonnes	Handbook on Fisheries Statistics 2022 (People's Archive of Rural India)
Number of KCC cards issued to fishers (2022-23)	1,42,458	Department of Fisheries, GoI (Press Information Bureau)
Investment in the fisheries sector (last 9 years)	Rs. 38,572 crore	Department of Fisheries, GoI (Press Information Bureau)
Increase in inland and aquaculture production (2022-23)	10 lakh tonnes	Department of Fisheries, GoI (Press Information Bureau)
Number of fishers enrolled under insurance scheme (2022-23)	33.21 lakh	Department of Fisheries, GoI (Press Information Bureau)

Source:

1. Handbook on Fisheries Statistics 2022, Ministry of Fisheries, Animal Husbandry and Dairying, Government of India.

2. Department of Fisheries, Government of India Annual Report 2022-23.
3. Press Information Bureau (PIB) releases and updates on the fisheries sector.

Policy and infrastructure support

Effective policy and infrastructure support are crucial for the successful adoption of technology in Indian fisheries. Government initiatives such as subsidies, grants, and training programs can alleviate financial and knowledge barriers. The development of robust infrastructure, including reliable internet connectivity and access to modern equipment, is essential for facilitating technology uptake. Collaborative efforts between government, private sector, and non-governmental organizations can drive the widespread adoption of technology, leading to sustainable growth in the fisheries sector. The government can play a pivotal role by providing financial incentives and subsidies to offset the initial costs of adopting new technologies. These incentives can be targeted towards small-scale fishermen and aquaculture farmers who may otherwise lack the resources to invest in advanced equipment. Additionally, grants for research and development can spur innovation in fisheries technology, addressing specific challenges faced by the sector. Training programs and extension services are vital for building the technical capacity of fishermen, ensuring that they can effectively utilize new tools and technologies. Infrastructure development is another critical area. Ensuring reliable internet connectivity in coastal and rural areas enables the use of digital applications and online marketplaces. Investment in cold chain infrastructure and logistics can reduce post-harvest losses and improve the quality of seafood reaching consumers. Moreover, establishing technology hubs and incubators in key fishing regions can facilitate access to cutting-edge tools and foster collaboration among stakeholders.

Public-private partnerships can enhance the reach and impact of technology adoption initiatives. Private companies can bring innovation and efficiency, while non-governmental organizations can provide grassroots-level support and advocacy. These partnerships can help scale successful pilot projects and create sustainable models for technology integration in fisheries. By aligning policy measures with infrastructure development and collaborative efforts, India can unlock the full potential of technology in its fisheries sector, ensuring long-term economic and environmental benefits.

Research methodology

a. Type of data: The research utilizes secondary data. Secondary data includes academic journals, government reports, and industry publications.

b. Type of research: The research is exploratory, aiming to provide an in-depth understanding of the benefits and barriers of technology adoption in Indian fisheries.

c. Period of research: The research is conducted for one year, from January 2021 to December 2023.

Conclusion:

The adoption of technology in Indian fisheries offers numerous benefits, including increased productivity, improved sustainability, and enhanced socio-economic outcomes. However, significant barriers such as financial constraints, lack of awareness, and inadequate infrastructure hinder the widespread implementation of these technologies. Addressing these challenges through targeted policies, infrastructure development, and collaborative efforts can unlock the full potential of technology in the fisheries sector, ensuring sustainable growth and prosperity for all stakeholders. The government must play a proactive role in creating an enabling environment that supports technological innovation. Moreover, continuous capacity-building programs for fishermen and aquaculture farmers are essential to maximize the benefits of technology. Engaging local communities and fostering a culture of innovation can further drive the successful integration of technology in the fisheries sector, making it more resilient and competitive on a global scale.

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SUSTAINABLE FISHERIES MANAGEMENT IN INDIA: CHALLENGES AND OPPORTUNITIES

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Abstract:

Sustainable fisheries management in India is critical for ensuring long-term ecological balance, food security, and economic stability. This paper examines the current challenges and opportunities in the sector, focusing on policy frameworks, technological innovations, community engagement, and ecological impacts. The research highlights key strategies for improving sustainability and offers recommendations for policymakers, stakeholders, and practitioners. The analysis draws on recent data and case studies to comprehensively understand the dynamics. Emphasising the need for an integrated approach, this study underscores the importance of harmonising environmental, social, and economic objectives. Effective management practices are essential for preserving marine biodiversity, ensuring the livelihoods of fishing communities, and meeting the growing demand for fish products sustainably.

Keywords: Sustainable Fisheries, India, Fisheries Management, Challenges, Opportunities, Ecological Impact, Policy Framework.

Introduction:

Fisheries play an important role in India's economy, providing a living for millions and considerably contributing to food security. However, the sector confronts various issues, such as overfishing, habitat damage, climate change, and ineffective policy implementation. Sustainable fisheries management seeks to solve these difficulties by supporting techniques that preserve fish stocks' long-term survival, conserve marine ecosystems, and improve the socioeconomic well-being of fishing communities. This paper explores the multifaceted aspects of sustainable fisheries management in India, identifies key challenges, and highlights potential opportunities for improvement. India's vast coastline of over 7,500 kilometres and extensive inland water resources offer significant potential for fisheries development. The sector includes marine, coastal, and inland fisheries, all of which present unique difficulties and opportunities. Overfishing has caused the extinction of some fish species, endangering biodiversity and livelihoods that rely on them. Additionally, industrial, agricultural, and domestic pollution has further degraded aquatic habitats, complicating conservation efforts. Climate change poses another severe threat, altering water temperatures, sea levels, and ocean currents, which affect fish migration patterns and breeding cycles. These changes need adaptive management systems that can adjust to changing environmental conditions. Furthermore, there is a pressing need to strengthen policy frameworks and improve enforcement to address illegal, unreported, and unregulated (IUU) fishing activities. Effective sustainable fisheries management requires a collaborative approach involving government agencies, research institutions, non-governmental organisations, and local communities. By integrating traditional knowledge with modern scientific techniques, India can develop robust strategies to ensure the sustainability of its fisheries.

Review of literature:

Smith *et al.* (2022) explored the impact of climate change on fish stocks in India, emphasising the need for adaptive management strategies. The study highlighted that rising sea temperatures and changing ocean currents have significantly affected fish distribution and abundance, necessitating a shift in traditional fisheries management practices. Additionally, the research underscored the importance of incorporating climate models into fisheries management to predict future trends and mitigate potential impacts. The authors also suggested that international collaboration could enhance the effectiveness of these adaptive strategies. Rao (2022) examined the role of community-based fisheries management in India. The research found that involving local fishing communities in decision-making led to better compliance with regulations and more sustainable fishing practices. The study emphasised the importance of empowering local communities through education and capacity-building programs. Furthermore, Rao highlighted the success of several community-led initiatives in reducing overfishing and preserving marine biodiversity. The study also pointed out that such community involvement can lead to improved social cohesion and economic resilience in fishing communities. Gupta and Sharma (2023) analyzed the effectiveness of policy frameworks in promoting sustainable fisheries in India. They identified gaps in the implementation of existing policies and recommended the adoption of more integrated and holistic approaches to fisheries management, including stronger enforcement mechanisms and better coordination between various stakeholders. The study revealed that policy fragmentation and lack of enforcement have led to significant challenges in achieving sustainability. Gupta and Sharma also discussed the need for more comprehensive data collection and monitoring systems to inform policy decisions. Furthermore, the authors called for increased funding and resources to support the implementation of sustainable practices, emphasizing the role of government and non-governmental organizations in this endeavour.

Objective of the paper

The paper's objective is to critically analyse the current state of fisheries management in India, identify the primary challenges hindering sustainability, and explore the opportunities available for promoting sustainable practices. This paper aims to provide actionable recommendations for policymakers, stakeholders, and practitioners to enhance the sustainability of the fisheries sector in India.

Policy framework and governance

Effective policy frameworks and governance are critical to sustainable fisheries management. This section discusses the existing policies related to fisheries in India, their implementation challenges, and the need for reforms. It also looks at the function of governance structures in maintaining compliance and encouraging sustainable practices. India's fisheries sector is governed by a complex web of policies at the national and state levels. The Marine Fisheries Regulation Act (MFRA), implemented by coastal states, aims to regulate fishing activities within territorial waters. Additionally, the National Policy on Marine Fisheries, 2017, provides a comprehensive framework for sustainable development, conservation, and management of marine fisheries resources. Despite these policies, implementation remains a significant challenge due to inadequate enforcement mechanisms, lack of coordination among various government agencies, and insufficient funding. Moreover, overlapping jurisdictions and fragmented regulations often lead to inconsistencies and gaps in policy enforcement. The absence of a unified legal framework further complicates efforts to manage fisheries sustainably. There is a pressing need for reforms that streamline regulatory processes, enhance inter-agency collaboration, and allocate adequate resources for enforcement and monitoring. Strengthening institutional capacity and improving transparency and accountability in governance structures are also essential to ensure compliance with regulations. Furthermore, integrating traditional knowledge with modern scientific approaches can enhance policy effectiveness and foster more inclusive governance. Engaging local communities in policy development and implementation processes can lead to better compliance and more sustainable outcomes. By overcoming these governance concerns, India can build a more resilient and sustainable fisheries sector that benefits the environment and the lives of fishing communities.

Technological innovations and sustainability

Technological innovations have the potential to revolutionize fisheries management. This section examines the various technologies being used in India, such as satellite monitoring, electronic logbooks, and mobile applications, and their impact on sustainability. It also highlights the challenges in adopting these technologies and the importance of capacity-building among stakeholders. Satellite monitoring systems enable real-time tracking of fishing vessels, helping to prevent illegal, unreported, and unregulated (IUU) fishing. These systems provide valuable data on fishing patterns, vessel movements, and potential violations, allowing authorities to take timely action. Electronic logbooks streamline the reporting process, making it easier for fishers to record their catches and for authorities to monitor and manage fish stocks effectively. Mobile applications facilitate communication between fishers and regulators, provide access to market information, and offer educational resources on sustainable fishing practices.

Despite these advancements, the adoption of new technologies faces several challenges. High costs and lack of infrastructure can limit access to these tools, particularly for small-scale fishers. Additionally, there may be resistance to change due to a lack of awareness or understanding of the benefits of technological innovations. To overcome these barriers, it is crucial to invest in capacity-building initiatives that educate stakeholders on the use and advantages of new technologies. Providing financial support and incentives can also encourage wider adoption and integration of these tools into everyday fishing practices. Moreover, collaborative efforts between government agencies, research institutions, and private sector partners can drive technological advancements and ensure their effective implementation. By leveraging technology, India can enhance the sustainability of its fisheries sector, improve resource management, and support the livelihoods of fishing communities.

Community engagement and education

Engaging local communities and providing education are essential for the success of sustainable fisheries management. This section explores the role of community-based management approaches, the importance of involving fishers in decision-making processes, and the benefits of education and awareness programs in promoting sustainable practices. Community-based fisheries management (CBFM) empowers local fishers to take an active role in managing their resources. By involving communities in decision-making processes, CBFM fosters a sense of ownership and responsibility, leading to better compliance with regulations and more sustainable fishing practices. Successful examples of CBFM in India have shown that local knowledge and traditional practices can complement scientific approaches to resource management. These initiatives often result in improved fish stock health, enhanced biodiversity, and increased economic benefits for fishers. Education and awareness programs play a crucial role in promoting sustainable fisheries. These programs can raise awareness about the importance of conservation, teach sustainable fishing techniques, and provide information on regulations and best practices. Workshops, training sessions, and informational campaigns can help fishers understand the long-term benefits of sustainable practices and encourage them to adopt more responsible behaviours. Additionally, educational initiatives can address knowledge gaps and equip fishers with the skills needed to use new technologies effectively. Moreover, involving women and youth in educational programs can have a multiplier effect on community engagement and sustainability. Women, who often play key roles in post-harvest activities and household decision-making, can be powerful advocates for sustainable practices. Engaging youth ensures the transfer of knowledge to future generations, fostering a culture of sustainability within fishing communities. Building partnerships between government agencies, non-governmental organizations, and local communities can enhance the effectiveness of education and engagement efforts. These partnerships can provide the resources and support needed to implement comprehensive programs that address the unique needs and challenges of different communities. By prioritizing community engagement and education, India can create a more sustainable and resilient fisheries sector that benefits both the environment and the livelihoods of its people.

Research methodology

a. Type of data

The study utilises secondary data. Secondary data is sourced from existing literature, government reports, and international studies on fisheries management.

b. Type of research

The present research is descriptive.

c. Period of research

The research is conducted over one year, from January 2022 to December 2023.

Conclusion:

Sustainable fisheries management in India faces significant challenges, including overfishing, climate change, and policy implementation gaps. However, there are also numerous opportunities for improvement, such as the adoption of advanced technologies, community engagement, and policy reforms. By addressing these challenges and leveraging the available opportunities, India can ensure the long-term sustainability of its fisheries sector, thereby supporting the livelihoods of millions and contributing to food security and economic stability. A multifaceted approach that combines scientific research, traditional knowledge, and innovative practices is essential for achieving sustainability goals. Policymakers must prioritize the enforcement of regulations and provide adequate funding to support sustainable initiatives. Strengthening the collaboration between various stakeholders, including government agencies, local communities, and the private sector, can lead to more effective resource management. Furthermore, continuous monitoring and adaptive management are crucial to respond to changing environmental conditions and emerging challenges. By fostering a culture of sustainability and resilience, India can protect its rich marine biodiversity and secure a prosperous future for its fishing communities.

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REIMAGINING THE 3RS: A REVIEW OF MODERN STRATEGIES AND INNOVATIVE APPROACHES IN ANIMAL RESEARCH

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Abstract:

The 3Rs principle, consisting of Replacement, Reduction, and Refinement, has become a crucial guideline for using animals and alternatives in scientific research. This review explores modern strategies and innovative approaches that adhere to the 3Rs while enhancing scientific rigor and animal welfare. Current animal research practices encompass various applications, including biomedical studies, veterinary medicine training, ecological research, and translational biomedical research. The growing emphasis on systematic reviews, meta-analyses, and open science practice aims to improve the validity and translational potential of experimental studies. The EU Directive calls for the complete substitution of animal testing with scientifically validated alternative methods, such as in silico and in vitro techniques. Integrated strategy frameworks that combine various methods and information sources have been shown to partially or fully replaced animal experimentation. The FDA/CDRH has released guidance documents suggesting the refinement, minimization, or substitution of animal testing methods to enhance conventional rodent testing. New Approach Methodologies (NAMs), including *in silico, in chemico, in vitro*, and *ex vivo* techniques, align with ethical concerns and 3Rs principles. The biotechnology field presents new scientific challenges, enabling scientists to evaluate biological risks without using animals, such as the use of Environmental DNA (eDNA) research in aquatic ecosystems. By prioritizing animal replacement, raising awareness of available alternative methods, and promoting education and training, the scientific community can advance the vision of more ethical, cost-effective, efficient, accurate, and humane research practices.

Keywords: 3Rs, Replacement, Reduction, Refinement, Animal Research

Introduction:

The 3Rs, which consist of Replacement, Reduction, and Refinement, were first introduced in 1959 and have since become essential principles guiding the use of animals and alternatives in scientific research. This article presents a current perspective on implementing 3Rs by recognizing the importance of modern technology and demonstrating that adhering to 3Rs can enhance both scientific rigor and animal welfare. This modern methodology emphasizes the importance of the 3Rs, relocating them from an ethical standpoint where they were frequently dismissed as an undesirable responsibility. Recent instances illustrate the potential to conduct superior science by employing 3Rs technologies, which provide faster, more reliable, and cost-effective outcomes (MacArthur Clark, 2018). Certainly, techniques that utilize replacement methods can facilitate discoveries that might not otherwise be possible with the use of animals. These approaches are often more adaptable and efficient because compliance with regulatory oversight requirements is generally streamlined. Although it is widely acknowledged that strict oversight is necessary, it is essential to ensure that the accompanying bureaucracy is not so burdensome that scientists are deterred from conducting justifiable and significant research involving animals. Public support for research is contingent on ensuring that animals are not subjected to unnecessary suffering and that significant benefits accrue from research. Simultaneously, society actively seeks innovative medical and scientific advancements that can only be achieved through research. Therefore, it is essential to strike a balance between safeguarding animal welfare and enabling high-quality science. Establishing and maintaining this equilibrium is crucial for fostering and preserving public confidence in the acceptability and responsible management of animal-based research.

Current approaches to animal research

Current animal research practices encompass various applications, including biomedical studies, veterinary medicine training, ecological research, and translational biomedical research. Laboratory animals are crucial for testing hypotheses, developing new drugs, and ensuring product safety (Zanatto *et al.*, 2024). Although ethical concerns and the aim of reducing animal stress have driven the development of alternative approaches and the promotion of noninvasive research techniques in the field of wildlife biology, these methods have not been widely adopted (Rajathy Port Louis *et al.*, 2018). The growing emphasis on systematic reviews, meta-analyses, and open science practice is aimed at improving the validity and translational potential of experimental studies. These approaches are designed to enhance research transparency, quality, and public trust in biomedical research (Zemanova, 2020). Diverse methods play a crucial role in advancing scientific understanding while upholding ethical principles and ensuring animal welfare. By concentrating on the 3Rs principle, which encompasses replacement, reduction, and refinement, these approaches contribute to the humane treatment of animals in scientific research.

1. Replacement strategy

The EU Directive calls for the complete substitution of animal testing with alternative methods that have been scientifically validated and do not involve the use of live animals (Eskes, 2022). To attain this objective, multiple strategies have been implemented, such as in silico and in vitro techniques, including molecular docking and cell culture testing, which are both cost-effective and consistent with the 3Rs principle (Huang *et al.*, 2021a). Utilizing integrated strategy frameworks that combine various methods and information sources has been shown to partially or fully replace animal experimentation, thereby making animal research a last-resort option (Eskes, 2019). The goal of these methods is not only to decrease the number of animals used in research, but also to improve the scientific validity of the results by delivering reliable and reproducible findings. This contributes to the ethical advancement of animal research.

Alternatives are methods that either completely replace animals or reduce their usage in procedures, thereby reducing pain and suffering. Millions of animals are used in biomedical research to evaluate drug safety and efficacy, which cause significant pain and distress. Researchers, organizations, and institutions worldwide are working to develop and validate alternative methods that are ethical, cost-effective, efficient, accurate, and humane. However, it is crucial for the scientific community to prioritize animal replacement over refinement and reduction strategies. By raising awareness of the available alternative methods and promoting education and training, we can advance this vision. Stricter regulations and scientifically validated alternatives can lead to a continued decline in the number of animals used in research, testing, and education in the future (Yadav & Singh, 2021).

The FDA/CDRH has released guidance documents suggesting refining, minimizing, or substituting animal testing methods to enhance conventional rodent testing. These recommendations are in accordance with the FDA Safety and Innovation Act Section 907, which enables rapid access to breakthrough therapies. Specifically, the FDA's Guidance for the Use of International Organization for Standardization 10993-1 allows large animal safety studies to be considered as an alternative to rodent tests, provided that scientific principles, methods, and endpoints (SPME) are applied. This guidance also includes study designs that employ methods for a more thorough examination of animal systems (Hampshire & Gilbert, 2019). The demand for experimental animals in the pharmaceutical and medical device industries is expected to rise owing to research and development. However, the use of these animals has drawn criticism due to suffering, death, and issues such as lack of trained labor, long protocols, high costs, and physiological relevance. To address these concerns, New Approach Methodologies (NAMs) are employed in toxicology and risk assessment, which aim to replace, refine, or reduce animal use. These methods, which include *in silico, in* *chemico*, *in vitro*, and *ex vivo* techniques, align with ethical concerns and 3Rs principles. However, each method has its own advantages and drawbacks (Kasoju & Kripasagari, 2024).

Clinical animal testing is crucial for assessing hypotheses about living creatures and for enhancing health and well-being. However, animal testing raises ethical concerns that have sparked debates and calls for alternative methods. Biotechnology presents new scientific challenges, enabling scientists to evaluate biological risks without using animals. For instance, the use of Environmental DNA (eDNA) research in aquatic ecosystems allows researchers to study invasive marine species without harming fish populations. Additionally, advanced analyses of environmental chemistry and virtual simulation models could potentially replace the need for animal testing. The obstacles presented by biotechnology provide a promising avenue for future progress and a promising outlook for comprehending and addressing the intricate relationship between instincts and culture (Dopico & Garcia-Vazquez, 2017). Simulations using human cells or animal-derived organs, tissues, or cells can provide cost-effective solutions for rapid toxicity and efficacy testing. However, these methods are limited in their ability to accurately replicate the intricate interactions between human organs, biological responses to specific administration routes, and toxicity of substances resulting from metabolic processes. New technologies such as organoids and organ-on-chips are being developed to address these limitations and efficiently evaluate the efficacy of drugs and nanoparticles. This could lead to the development of patient-specific treatments. Although these technologies show promise, it remains challenging to replace traditional animal experiments completely. However, the range of methods available to replace animal testing is expanding, suggesting the potential for refining, reducing, and ultimately phasing out animal testing (Jin *et al.,* 2020).

The primary objective of FRAME is to eradicate the utilization of laboratory animals in scientific and medical research, as indicated by its complete moniker, the Fund for the Replacement of Animals in Medical Experiments. Compared to reduction and refinement, replacement is the most vital and difficult of the 3Rs principles described by Russell and Burch. The ATLA journal, which focuses on non-animal alternatives, has recently featured articles that emphasize the progress made in this field. A comprehensive study was conducted to evaluate the effectiveness of a human corneal epithelium model for eye irritancy testing, demonstrating its potential as an alternative to rabbit testing based on the OECD guidelines. This study highlights the necessity of animal-independent research due to ethical concerns regarding fetal bovine serum. The Lush Prize provides funding for replacement research, and FRAME's yearly events promote advancements in human in silico trials for drug safety and effectiveness assessments (Trigwell, 2019).

Animal medicine, a vital component of Chinese medicine, faces a dilemma owing to the increasing industrial demand and scarcity of rare and endangered medicinal animals. This depletion impacts clinical demand and leads to ecological damage. Relying solely on artificial breeding to address this issue is insufficient. The challenge lies in preserving animal resources while satisfying both clinical and industrial needs using available medicines. It is essential to replace animal medicines with alternatives that possess similar chemical characteristics, efficacy, and safety to modernize Traditional Chinese Medicine (Zhu *et al.*, 2022). The challenges faced in screening inhaled compounds for lung diseases, safety testing, and environmental toxicant assessment are mainly the absence of appropriate animal models and restrictions on inhalation exposure in animals. Despite these obstacles, there are in silico models for aerosol characterization and deposition, as well as cellular and tissue models that can anticipate human effects. Rodent cells are typically used in the construction of these systems for validation in animal models. Alternatively, human data can be used to confirm the outcomes of experiments involving human cells. In terms of lung morphology, physiology, and administration, pulmonary exposure exhibits a greater degree of disparity between animals and humans than parenteral administration. Using a combination of in vitro techniques for aerosol characterization, advanced cell and tissue models, and in silico modeling, it is possible to replace animal studies in screening for inhaled drugs for the local treatment of pulmonary diseases. While animal studies may still be necessary for pulmonary diseases that have systemic effects, such as viral infections and lung cancer, the information obtained from advanced cellular models and in silico techniques can help reduce the extent of animal testing required (Fröhlich, 2021).

Evaluation of toxicity is essential for comprehending the harmful consequences of chemical substitutes, including nanomaterials and nanoparticles. However, it is necessary to consider animal welfare and ethics during the manufacturing process. The adoption of the 3Rs principle has resulted in the development of alternative methods to replace traditional animal tests, such as in vitro cell-based assays, tissue engineering, in silico structure-based techniques, and QSAR studies. These alternatives have been developed to minimize animal testing and promote ethical practices. These techniques prevent unethical experiments and are applicable to drug design and clinical purposes. In silico modeling combines in vitro experimental techniques to explore the mechanisms of newly developed products. Accurate toxicological outcomes depend on variables such as particle size, surface characteristics, aggregate size, and solubility. To effectively predict nanotoxicity, it is important to conduct a comprehensive analysis of the molecular and cellular mechanisms involved. Utilizing multidisciplinary approaches, such as in vitro and in silico experimental models, can help ensure consistent results and avoid contradictions (Huang *et al.*, 2021b). The European

Parliament has recommended a change in the health research approach, promoting human biology as a benchmark. The resolution suggests redirecting 10% of the EU's yearly research budget towards non-animal research utilizing non-animal models (NAMs) and cutting down animal use by 200,000 annually. This plan would halt animal use in approximately 30 years while preserving the EU's prosperous research atmosphere. The aim is to establish a scientific environment where NAMs are embraced as the new standard, researchers possess the necessary competencies, and research is centered on human biology. This aligns with the desires of European citizens and benefits drug discovery and development. The proposed strategy offers a mutually beneficial outcome for both science and animals (Marshall *et al.,* 2022).

The employment of animals in research, including preclinical testing and drug experimentation, has drawn criticism and opposition from activists and anti-vivisectionists. In the mid-1990s, this issue reached its zenith, but has since waned, as other matters, such as climate change, have assumed greater prominence. Protests at Oxford University during the construction of its Biomedical Sciences Building, which housed primates, sparked a fury of anti-vivisectionists in the United Kingdom. These demonstrations led to arson attacks, bomb threats, and the mistreatment of scientists, resulting in injunctions against animal rights groups. Despite this, the public's support for animal testing was waning, as the 10% success rate on animals had a significant impact on human health. Consequently, politicians, regulatory agencies, and funding agencies have urged scientists to minimize the use of mammals in research based on the 3R principle (Hunter, 2023).

One study emphasized the increasing application of ethical principles that were typically reserved for human research to nonhuman animals, with a focus on the translation of animal research findings into human clinical benefits. This paper contributes an epistemological foundation to complement existing practical and ethical arguments by advocating the acknowledgement of animal dissent. Possible ways to address animal dissent include overcoming it, training animals to bypass it, or significantly modifying research practices to respond to it. Although difficult, the latter option provides the most comprehensive solution aligned with ethical, practical, and epistemological considerations. Acknowledging and addressing animal dissent could result in significant changes to biomedical research practices, ultimately improving the ethical treatment of animals and the validity of research findings (Johnson, 2023).

2. Reduction strategy

The 3Rs approach is designed to optimize statistical power by minimizing the number of animals used; however, this approach may be at odds with the objective of enhancing information content without expanding the number of animals involved (“Ten Strategies to

Increase Information (and Reduce Sample Size)," 2023). Innovative technologies, such as in silico simulation, informatics, 3D cell culture models, and organ-on-chips, can significantly reduce the need for animal sacrifice in biomedical research. Instead of animals, scientists have developed animal-friendly affinity reagents and direct human body investigations for treatment. Electronic health records can also aid in reducing the number of animals used in experiments. However, the reliability of animal experiments in predicting human health outcomes remains questionable. Although alternatives to animal experiments can decrease the use of animals, they cannot completely eliminate their need. It is expected that animals will be used wisely in teaching and research, and their importance in life sciences will not be overlooked (Rai & Kaushik, 2018).

Previous research has demonstrated that incorporating historical controls in biomedical experiments can both ethically and practically decrease the number of current animal subjects without diminishing statistical power. This can be achieved using traditional t-tests, contrasts, or mixed models. According to simulations, the inclusion of historical controls in the analyses can reduce the number of current control subjects by more than half, leading to a significant reduction in the number of animal subjects, and consequently, time and money (Kramer & Font, 2016). A study was conducted to investigate how the principles of 3Rs are applied in toxicological research within the pharmaceutical industry, with a focus on minimizing the number of animals used in both regulatory and investigatory in vivo experiments. The study demonstrated that substantial reductions in animal use were attained through approaches such as enhanced study design, method development, and project coordination. This study emphasizes the significance of fostering a robust 3R culture, with scientific engagement, collaboration, and responsive management as indispensable aspects. Advocating a commitment to 3R leadership is suggested, which should entail the participation of various departments and professional groups in innovation, validation, and implementation projects. Rigorous synergies among the 3Rs have emerged, leading to the inference that in silico, in vitro, and in vivo methods collectively exhibit the capacity to strategically employ reduction R (Törnqvist *et al.*, 2014).

Additionally, intra-experimental reduction offers the greatest potential for reducing the number of animals used in experiments. This can be achieved by improving the experimental design and statistical analysis at the individual level. In contrast, supra-experimental reduction aims to minimize animal use by altering the context in which the experiments are conducted. This approach involves enhancing education and training, reducing the number of surplus animals, analyzing test specifications, and maximizing animal reuse. Enhanced experimental reduction refers to innovative advancements built upon existing developments by refining research or production methods to improve quality, consistency, and safety. A revised definition of reduction has been suggested, which excludes the level of detail needed, as some instances may still be deemed acceptable (De Boo & Hendriksen, 2005). Animal models have played a crucial role in medical advancements. However, there is often an excess of material that can be utilized by other researchers. To make this resource more readily available, the SEARCH framework was designed to facilitate the sharing and coordination of animal holdings. The SEARCH framework aims to increase the accessibility of animal research materials to the scientific community in biomedical studies. By fostering collaboration between journals, funding agencies, and researchers, this framework advocates for a more efficient use of resources by prioritizing the utilization of existing animal models and samples before generating new ones (Morrissey *et al.*, 2017).

The Environmental Protection Agency is working on creating New Approach Methods (NAMs) to evaluate chemicals for their health effects, with the aim of decreasing reliance on animal testing and safeguarding both human health and the environment. Non-vertebrate animal technologies, methods, and approaches, often referred to as NAMs, are employed in chemical hazard and risk assessments. These include in vitro tests, chemico-assays, and in silico models. These NAMs are functionally equivalent to alternatives to mammalian testing (Introduction and Historical Overview, n.d.).

3. Refinement strategy

Refinement techniques in animal studies, as detailed in numerous research papers, concentrate on reducing pain and discomfort in laboratory animals while improving the quality of the obtained data. The 3Rs principle plays a crucial role in this regard, aiming to enhance animal well-being and research outcomes. Research has demonstrated that European research groups, with financial support, particularly in the UK, have made significant advancements in refinement protocols, including improvements in experimental procedures and housing conditions (Łaz *et al.*, n.d.)(Fröhlich & Loizou, 2023). Furthermore, it has been emphasized that the standardization of environmental enrichment techniques is necessary to guarantee the consistency of research samples and minimize the utilization of animals for experimental objectives (FISCHER *et al.*, 2021). In addition, the Animal Study Registry has been implemented to foster transparency, reproducibility, and animal well-being in bioscience research by mandating the registration of animal studies (Bert *et al.*, 2019a).

A comprehensive review was carried out to summarize the advancements in refinement protocols made by European Union-based research groups from 2011 to 2021, with a particular emphasis on the improvements in experimental procedures for mice. Over two-thirds of the studies received financial support, with 26 (mostly British) receiving national-level funding and 8 receiving European Union-level funding. The findings indicate a clear commitment by the scientific community to enhance the welfare of laboratory animals, despite the fact that funding was not always specifically designated for this purpose. However, more targeted financial support at both national and European Union levels is necessary. The data imply that countries that invest in refinement have higher success rates in publishing refinements, suggesting that greater progress could have been made in refinement during this period. (Díez-Solinska *et al.,* 2022).

The Animal Study Registry (ASR) was introduced in January 2019 to heighten transparency and reproducibility in bioscience research while promoting animal welfare. It is a complementary resource designed to facilitate exploratory and confirmatory studies in applied science, as well as basic and preclinical research. The registration form was comprehensive, asking researchers detailed questions about their study design, methods, and statistical analyses. Upon registration, studies are automatically assigned a digital object identifier (DOI), and researchers can restrict access to their work for up to five years to safeguard their intellectual property. At the conclusion of the embargo period, the complete study became publicly accessible. The ASR is securely embedded in the German Federal Government's infrastructure, ensuring continuity and data security (Bert *et al.,* 2019b).

A previous study emphasized the significance of the 3R-Refinement protocol, which seeks to enhance the well-being of laboratory animals, while improving the scientific validity of research outcomes. This study underscores the importance of providing enriched housing environments, implementing nonrestraint handling methods, refining dosing and sampling techniques, ensuring optimal pain management, and conducting regular animal welfare assessments. It also highlights the advantages of collaborating with the animal care and ethics committees. The 3R-Refinement protocol offers several benefits, including improved animal welfare, enhanced research quality, reduced variability, and positive feedback from researchers and animal care staff. This study also suggests ways to promote the adoption of the protocol, such as sharing best practices, conducting training programs, and partnering with regulatory bodies. This emphasizes the need to align scientific progress with ethical considerations and build a more compassionate and responsible future for animal research (Rinwa *et al.,* 2024).

The central theme of "good welfare equals good science" has been a significant focus of research since Trevor Poole first established the connection between animal welfare and the quality of scientific outcomes. Animal welfare refers to an animal's ability to cope with its living conditions, including its health, comfort, well-being, safety, natural behavior, and the absence of negative states such as pain, fear, and distress. While it is true that some scientific studies may require some degree of animal suffering to achieve their objectives, minimizing unnecessary harm is essential. Compromised welfare can lead to abnormal behavior, physiology, and immunology, resulting in unreliable conclusions and unwanted variability in scientific output, which can negatively impact experiment reliability and repeatability. To further emphasize the importance of this link, NC3Rs are working to refine animal research (Prescott & Lidster, 2017). Moreover, the implementation of advanced computational resources has enabled a novel approach for portraying intermolecular interactions in molecular dynamics simulations. Instead of employing a limited number of interatomic springs, the present method considers all atom-atom pair interactions between neighboring molecules to calculate the MC energy. The values of the spring constants, Ki, are determined through an empirical formula that considers the pair type and separation distance, and these parameters can be adjusted by fitting. This technique was demonstrated using paracetamol polymorphs (Welberry, 2022).

Global legislative impact

The 3Rs principle, comprising Replacement, Reduction, and Refinement, has had a substantial impact on animal research regulations worldwide. First introduced in European directives in the 1980s and further developed in subsequent legislation, the 3Rs aim to enhance animal welfare, minimize the number of animals used, and improve experimental conditions. Despite its widespread adoption, recent studies show a lack of comprehensive reporting on 3Rs approaches in preclinical animal research, suggesting a need for increased education, interdisciplinary collaboration, and funding initiatives to promote the effective implementation of 3Rs principles. Establishing internal 3Rs advisory groups within organizations has been proposed as a strategy to drive innovation, accelerate technical development, and ensure the ethical application of the 3Rs in animal experimentation, ultimately contributing to the advancement of scientific practices while prioritizing animal welfare.

The regulation of animal models that adhere to the 3Rs principle in the European Union is governed by Directive 2010/63/EU. The scientific and technological advancement of European legislation is ensured and adapted through the 66 articles and eight annexes that make up this legislation. This legislation replaced the Council Directive of November 24, 1986 (86/609/EEC) (CELEX_31986L0609_EN_TXT, n.d.). Each state is required to implement Directive 2010/63/EU on the protection of animals used for scientific purposes, and Italy must comply specifically with Legislative Decree 26/2014 (Eli_dir_2010_63_oj_IT_TXT, n.d.). Since the passage of two directives 24 years apart, the number of acquaintances in the field of laboratory animal science has greatly increased. As a result, it has become necessary to update the legal framework. The directive is not intended to be a set of guidelines that either encourages or restricts the use of animals in research, but rather to safeguard the welfare of animals that are utilized in research facilities. The main objective of the directive is to supply comprehensive specifications that minimize discrepancies among member states' methods for ensuring the well-being of laboratory animals, creating a more homogeneous European environment. The value of 'Animal Welfare' is explicitly stated in Article 13 of the Treaty on the Functioning of the European Union, making it a fundamental principle of the European Union." (4b17a07e2, n.d.).

The European directive in question has made one of the most significant updates, including the assessment of pain and distress in animals used for scientific purposes, which has not been previously addressed in Directive 86/609/EEC. In accordance with Annex VIII, "The severity of the procedure is determined based on the level of pain, suffering, distress, or prolonged damage to which the individual animal is presumably subjected during the procedure itself" (Eli_dir_2010_63_oj_IT_TXT, n.d.).

According to Article 26 of European Regulation 63/2010, another significant innovation is highlighted, "that each breeder, supplier, and user sets up an Animal-Welfare Body (AWB)". Each AWB comprises a designated individual responsible for the well-being and care of animals, a veterinarian, and, in the case of a facility authorized for experimentation, a scientific member who serves as a guarantor of scientific quality. The position of the biostatistician in the AWB remains a subject of ongoing debate and discussion (Grignaschi *et al.,* 2018); however, ensuring that the study design aligns with the reduction principle of the 3Rs is critical.

Conclusion:

Reimagining animal research with respect to the 3Rs necessitates not only enhancing current practices but also adopting novel approaches that extend the limits of feasibility. Innovative strategies for reduction, refinement, and replacement coupled with emerging alternatives are transforming the landscape of animal research. By persistently advancing these initiatives, the scientific community can uphold ethical standards while propelling progress in biomedical research. The ultimate objective is to establish harmony in which scientific inquiry is pursued with the utmost regard for animal welfare, resulting in improved outcomes for both science and society.

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GENE EDITING FOR IMPROVEMENT OF ANIMAL TRAITS AND DISEASE RESISTANCE

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Abstract:

Gene editing technology is transforming genetic and biological research by enabling precise modifications to the genomes of animals, significantly advancing our understanding of gene functions and their applications in improving animal traits. This chapter explores various genome editing tools, including CRISPR/Cas9, Zinc Finger Nucleases (ZFNs), and Transcription Activator-Like Effector Nucleases (TALENs), highlighting their role in enhancing growth rates, improving feed efficiency, and increasing disease resistance in livestock. The potential for gene editing extends to the development of disease-resistant breeds, enhanced milk production, improved meat quality, and superior wool characteristics. Furthermore, this technology offers solutions to pressing agricultural challenges such as emerging diseases and climate change, providing a pathway towards more sustainable and resilient animal production systems.

Keywords: Gene Editing, CRISPR/Cas9, Zinc Finger Nucleases (Zfns), Transcription Activator-Like Effector Nucleases (Talens), Animal Traits, Disease Resistance, Livestock Improvement, Genetic Modification, Biotechnology, Animal Agriculture.

Introduction:

Genome editing technology is revolutionizing genetic and biological research by enabling precise modifications to the genomes of living organisms. This ability is significantly accelerating the study of functional genomics, offering deeper insights into gene functions and their interactions. Since its emergence in the 1990s, genome editing has seen the development of various tools and techniques for targeted gene editing. These tools are instrumental in studying both simple and complex genomes, allowing researchers to manipulate specific genes, thereby uncovering their roles in health, disease, and development. (Tavakoli, *et al.*, 2021)

Key technologies in genome editing include Zinc Finger Nucleases (ZFNs), which work by using zinc finger proteins that recognize specific nucleotide triplets, combined with the FokI endonuclease to target specific DNA sequences; Transcription Activator-Like Effector Nucleases (TALENs), which bind to specific DNA sequences and induce double-strand breaks (DSBs), and are being used to improve animal traits and for genetic modifications (Jaya Bharati, 2020); and the CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)Cas9 (CRISPR Associated Proteins) system, which is revolutionizing genetic manipulation in large animals, enabling efficient, single-generation modifications to produce desirable traits for agriculture. This system is significantly advancing animal biotechnology and livestock breeding, allowing for precise improvements in disease resistance, meat quality, and animal welfare.

The potential applications of gene editing are vast. In livestock, gene editing techniques could lead to animals that grow faster, produce higher-quality meat or milk, and exhibit improved reproductive traits. Additionally, the development of disease-resistant breeds could reduce reliance on antibiotics, enhance animal welfare, and safeguard agricultural investments. For companion animals, gene editing offers the prospect of eliminating genetic disorders, enhancing overall health, and extending lifespans, thus improving the quality of life for pets and their owners alike.

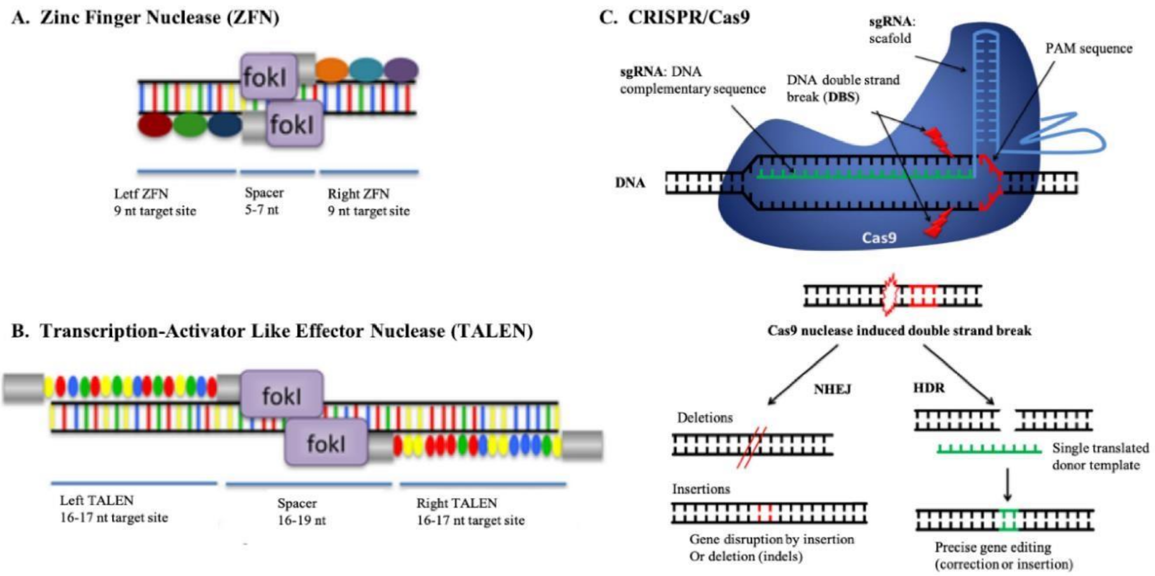

Fig. 1: Nuclease based genome editors

Source: https://www.mdpi.com/2076-2615/10/12/2236

The growing need for adaptability and rigidity in beast husbandry to ensure global food security and ameliorate mortal health. It emphasizes the challenges farmers face, such as emerging diseases, climate change, and dwindling natural resources. Conventional breeding programs form the backbone of animal genetic improvement, securing incremental, cumulative, and permanent genetic gains. These methods include selective breeding, hybridization, and chromosome set manipulation, as well as assisted reproductive techniques such as cloning, embryo transfer, and artificial insemination. These techniques are used to accelerate and amplify the rate of genetic gain in animal breeding programs. Biotechnologies, including genome editing (GnEd), allow the modification of phenotypes in ways that reduce the time and cost to achieve breeding goals.

(WrayCahen, 2022).

Improved animal traits

1. Increasing growth rate and feed efficiency

- **Selective breeding:** Animals that grow faster and convert feed into body mass more efficiently are chosen for breeding. This results in offspring that reach market size quicker and require less feed.
- **Genetic modification:** Techniques like CRISPR are used to edit genes related to growth and metabolism, enabling animals to grow faster with improved feed conversion ratios.
- **Optimized nutrition:** Tailored diets and supplements enhance nutrient absorption, promoting faster growth and more efficient feed use (Lu, *et al.*, 2024).

2. Enhancing milk production and composition

- **High-yielding breeds:** Breeding cows, goats, and other dairy animals for higher milk yield is a primary focus, leading to animals that produce more milk with each lactation cycle.
- **Improving milk composition:** Genetic selection and dietary adjustments increase the fat and protein content of milk, making it more nutritious and valuable for dairy products like cheese and yogurt.
- **Health and longevity:** By breeding for improved udder health and longevity, dairy animals maintain high production levels over more extended periods (Sammy K. Kiplagat, Genetic Improvement of Livestock for Milk Production, 2012).

3. Improving meat quality and reducing fat content

- **Marbling and tenderness:** Selective breeding focuses on improving the quality of muscle tissue, leading to meat that is more tender and has better marbling, enhancing flavour and texture.
- **Reducing fat content:** Genetic and nutritional interventions reduce the overall fat content in meat without compromising taste, catering to health-conscious consumers.
- **Promoting lean muscle growth:** By promoting lean muscle growth through genetic selection and optimized feeding, animals produce higher-quality meat with a better meat-tofat ratio.

4. Increasing egg production and improving egg quality

- **High-laying breeds:** Chickens and other poultry are selectively bred to increase the number of eggs they lay per year, significantly boosting productivity.

- **Enhancing egg quality:** Focus on eggshell strength, yolk colour, and nutrient content, including higher levels of Omega-3 fatty acids, improves egg quality. This is achieved through both breeding and dietary adjustments.
- **Improving hatchability:** In breeding operations, improving the fertility and hatchability of eggs ensures a more efficient and productive poultry operation (Pan K.L. *et al.*, 2023).

5. Improving wool quality and quantity

- **Selective breeding for fiber quality:** Sheep and other wool-producing animals are bred for finer and more uniform wool fibers, which are more desirable in the textile industry.
- **Increasing wool yield:** Breeding programs focus on increasing the amount of wool produced per animal, leading to higher productivity.
- **Enhancing wool strength and elasticity:** Genetic selection improves the tensile strength and elasticity of wool, making it more durable and versatile for various applications (Muhammad Jamshed Khan., 2012).

Disease resistance

Disease resistance in animals is a critical area of research that aims to improve health and productivity in livestock by enhancing their ability to withstand infections. Genetic resistance plays a pivotal role in this process, allowing certain breeds or individuals to naturally fend off diseases with fewer adverse effects. For example, in cattle, genetic selection has led to the development of breeds with inherent resistance to tick-borne diseases, such as babesiosis and anaplasmosis. Similarly, in sheep, selective breeding for resistance to gastrointestinal nematodes has significantly reduced worm burdens and associated health issues. These advancements not only improve animal welfare but also reduce the need for chemical treatments and enhance overall farm productivity (Morris *et al.*, 2010; Bishop & Morris, 2007).

Genetic resistance to viral diseases (e.g.-PRRP, Influenza)

Research into genetic resistance to viral diseases is crucial for addressing persistent and economically damaging pathogens. In livestock, particularly pigs, genetic resistance to Porcine Reproductive and Respiratory Syndrome (PRRS) is a key focus. PRRS, caused by PRRSV, results in severe reproductive and respiratory problems, leading to significant economic losses. Advances in genomics have identified important genetic factors, such as the Guanylate Binding Protein 5 (GBP5) gene, which significantly reduces viral load and improves survival in infected pigs. This finding offers the potential to breed PRRSV-resistant pigs, potentially mitigating the virus's impact on the swine industry. (Lunney *et al.*, 2020).

Genetic resistance to viral infections like influenza is a key focus in human medicine. Influenza viruses are known for causing seasonal epidemics and pandemics, with varying effects across different populations. Research has highlighted the significant role of host genetics in influencing susceptibility to influenza. Notably, polymorphisms in the IFITM3 (Interferon-Induced Transmembrane Protein 3) gene have been linked to increased resistance to severe influenza. IFITM3 helps inhibit the entry of influenza viruses into host cells, offering protection. Individuals with certain IFITM3 variants are less likely to experience severe illness. This insight not only improves our understanding of host-pathogen interactions but also paves the way for developing targeted therapies and personalized medicine for influenza. (Everitt *et al.*, 2012).

Genetic resistance to bacterial disease (e.g. Mastitis, Salmonellosis)

Genetic resistance to bacterial diseases in livestock offers a promising approach to enhancing animal health and minimizing economic losses. Mastitis, a major bacterial infection in dairy cows, causes inflammation of the mammary glands, reducing milk production and quality. Research has pinpointed genetic factors that affect resistance to mastitis, including variations in the CXCR1 gene, which is crucial for immune cell migration. Cows with certain CXCR1 genotypes show a stronger immune response to bacterial infections, leading to reduced mastitis severity and incidence. This genetic insight enables selective breeding of cows with natural resistance to mastitis, potentially improving herd health and decreasing the need for antibiotics. (Sharma *et al.*, 2015).

Genetic resistance to salmonellosis, caused by Salmonella species, is being investigated to improve poultry and livestock health and food safety. Salmonellosis leads to gastrointestinal and systemic infections, posing risks to both animals and food products. Research has identified key genes like NRAMP1 (Natural Resistance-Associated Macrophage Protein 1) that are crucial for resistance.

NRAMP1 enhances the immune system’s ability to fight Salmonella by aiding macrophages in destroying bacteria. Animals with specific NRAMP1 variants show better control of Salmonella infections, resulting in lower bacterial loads and reduced transmission. This genetic understanding is being applied to breeding programs to create livestock that are naturally resistant to salmonellosis, improving animal welfare and food safety. (Berthelot-Herault *et al.,* 2003).

Genetic resistance to parasitic disease (e.g. Ticks, Worms)

Genetic resistance to parasitic diseases, such as tick infestations and worm infections, is vital for improving livestock health, particularly in cattle and sheep. Ticks, which transmit diseases like babesiosis and anaplasmosis, cause significant harm to cattle and sheep, leading to morbidity and mortality. Certain cattle breeds, such as Bos indicus (Zebu), exhibit natural resistance to ticks due to traits like thicker skin, grooming behavior, and enhanced immune responses. In sheep, resistance to gastrointestinal nematodes (worms) is linked to immune mechanisms such as IgA antibody production and eosinophil activity, which help reduce worm burdens and enhance health and productivity. Understanding the genetic basis of resistance to these parasitic diseases is essential for developing sustainable control strategies. Selective breeding programs that enhance genetic resistance can decrease reliance on chemical treatments, mitigate the threat of drug-resistant parasites, and improve animal welfare and productivity, contributing to more resilient livestock populations. (Morris *et al.*, 2010) (Bishop & Morris, 2007).

Improved immune response and reduced inflammation

Enhancing immune response while minimizing inflammation is a key goal in animal health research, aiming to improve disease resistance and overall well-being in livestock. A balanced immune system is crucial; it must effectively combat pathogens without causing excessive inflammation, which can lead to tissue damage and chronic health issues. Recent studies underscore the importance of selective breeding and genetic modification in achieving this balance. For instance, in dairy cattle, certain genetic traits have been linked to improved immune function and reduced inflammatory responses. Cows with these traits show higher resistance to mastitis, a common and costly inflammatory disease of the mammary gland, by efficiently clearing infections while maintaining a moderated inflammatory response, thus reducing tissue damage and facilitating quicker recovery. Additionally, dietary interventions, such as the inclusion of omega3 fatty acids in pig diets, have been explored to modulate immune responses and control inflammation. Omega-3 fatty acids are known to help resolve inflammation, supporting health and preventing chronic inflammatory conditions. This dual approach of enhancing immune competence while controlling inflammation is critical for optimizing animal health, reducing reliance on antibiotics, and boosting productivity in livestock. (Rupp & Boichard, 2003) (Calder, 2013)

Gene editing techniques

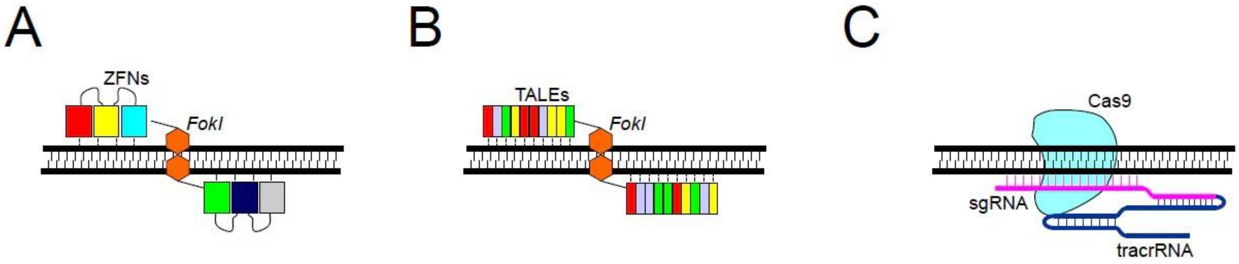

Fig. 2 : A) Zinc Finger Nucleases (ZFN), B) Transcription Activator-Like Effector Nucleases

(TALEN), & C) CRISPER/Cas9 Gene Editing

Source: https://www.ptglab.com/news/blog/crispr-cas9-talens-and-zfns-the-battle-in-geneediting/

CRISPER/Cas9:

The CRISPR/Cas9 system originates from the adaptive immune mechanisms found in bacteria and archaea. These microorganisms use CRISPR sequences to defend against viral infections by integrating fragments of viral DNA into their own genome. This system allows bacteria to "remember" past infections and mount a rapid defense against subsequent attacks by the same virus. When a virus infects a bacterium, the CRISPR locus captures and stores a piece of the viral DNA as a spacer sequence. During subsequent infections, the bacterium transcribes these stored sequences into CRISPR RNA (crRNA), which guides the Cas9 protein to the matching viral DNA, leading to its cleavage and neutralization. This discovery was crucial in developing CRISPR/Cas9 as a tool for precise gene editing in animals (Barrangou & Doudna, 2016; Jinek *et al.,* 2012; Knott & Doudna, 2018).

CRISPR/Cas9 technology, discovered as an adaptive immune mechanism in bacteria, has revolutionized genetic engineering with its precision and versatility. The system comprises the Cas9 protein, which acts as molecular scissors to create double strand breaks in DNA, and a guide RNA (gRNA) that directs Cas9 to specific genomic locations for targeted edits. This allows for precise modifications such as gene knockout, insertion, or correction, enabling significant advancements in research, medicine, and biotechnology. CRISPR/Cas9 is favored for its simplicity, cost-effectiveness, and ability to edit multiple genes simultaneously with minimal offtarget effects when well-designed. However, challenges remain, including potential off-target modifications, varying efficiency across cell types, and ethical concerns, especially in human germline editing. (Doudna & Charpentier, 2014) (Jinek *et al.*, 2012) (Hsu *et al.*, 2014) (Doudna & Charpentier, 2014; Cyranoski, 2015).

Components of the CRISPR/Cas9 system for gene editing in animals

The CRISPR/Cas9 system comprises several key components that are essential for its function in gene editing:

- Cas9 Protein: Cas9 is an endonuclease that acts as molecular scissors, cutting DNA at a specific location determined by the guide RNA. It has two nuclease domains, RuvC and HNH, that each cleave one strand of the DNA double helix, resulting in a double-strand break (Jinek *et al.*, 2012).
- CRISPR RNA (crRNA): This RNA molecule is derived from the spacers in the CRISPR locus and contains a sequence that matches the target DNA in the genome. The crRNA guides the Cas9 protein to the specific site in the DNA where the cut is to be made (Barrangou & Doudna, 2016).
- Trans-Activating CRISPR RNA (tracrRNA): The tracrRNA is essential for the maturation of crRNA and forms a complex with crRNA to guide Cas9 to the target

DNA. In engineered CRISPR systems, crRNA and tracrRNA are often fused into a single guide RNA (gRNA) to simplify the process (Deltcheva *et al.,* 2011).

- Protospacer Adjacent Motif (PAM): The PAM is a short DNA sequence that follows the target DNA sequence and is recognized by Cas9. The presence of a PAM sequence is crucial for the binding and cutting action of Cas9 (Anders *et al.,* 2014).
- Guide RNA (gRNA): In many applications, the crRNA and tracrRNA are combined into a single synthetic RNA molecule known as the guide RNA (gRNA). The gRNA directs Cas9 to the specific site in the genome that matches the 20-nucleotide sequence of the gRNA, adjacent to the PAM (Cong *et al.,* 2013).

These components work together to enable precise gene editing by introducing double strand breaks at specific locations in the DNA, which are then repaired by the cell's natural mechanisms, allowing for the insertion, deletion, or modification of genetic material in animal genomes (Knott & Doudna, 2018).

Mechanism:

1. Guide RNA (gRNA) design: A gRNA is designed to target a specific sequence in the animal's genome. (Jinek *et al.,* 2012)
2. Cas9 enzyme activation: The gRNA is complexed with the Cas9 enzyme, which is activated to cut the DNA. (Hsu *et al.,* 2014)
3. Double-strand break: The Cas9 enzyme cuts the DNA at the targeted sequence, creating a double-strand break. (Jinek *et al.,* 2012)
4. Repair machinery activation: The cell's repair machinery is activated to repair the break. (Hsu *et al.,* 2014)
5. Homologous recombination: The repair machinery uses a template to repair the break, allowing for precise editing of the genome. (Jinek *et al.,* 2012)

Transcription Activator-Like Effector Nucleases (TALEN):

TALEN (Transcription Activator-Like Effector Nucleases) is a gene editing technology that enables precise modifications to the genome. Developed as a versatile tool for targeted genetic manipulation, TALENs have been widely used in various organisms, including animals. This technique involves engineered nucleases that combine the DNA-binding properties of transcription activator-like effectors (TALEs) with the DNA-cleaving activity of the FokI nuclease. TALENs have been instrumental in advancing our understanding of gene function and developing animal models for research and therapeutic purposes (Joung & Sander, 2013).

The origins of TALENs can be traced back to the discovery of TALEs in plant pathogenic bacteria of the genus *Xanthomonas*. These bacteria use TALEs to bind specific DNA sequences in host plants, manipulating the plant's gene expression to facilitate infection. Researchers recognized the potential of TALEs to be reprogrammed to target specific DNA sequences in any genome. By fusing the DNA-binding domain of TALEs to the nonspecific cleavage domain of the FokI endonuclease, scientists created TALENs, which can introduce double-strand breaks at specific locations in the genome. This discovery has been a significant milestone in the field of gene editing (Christian *et al.,* 2010; Boch *et al.,* 2009).

Components of the TALEN system for gene editing in animals

The TALEN gene editing system comprises several key components:

- **Transcription Activator-Like Effectors (TALEs)**: TALEs are proteins derived from *Xanthomonas* bacteria that can be engineered to recognize specific DNA sequences. Each TALE contains a series of tandem repeats, where each repeat targets a single nucleotide in the DNA sequence. This modular structure allows TALEs to be customized to bind virtually any DNA sequence (Bogdanove & Voytas, 2011).
- **FokI nuclease domain**: The FokI nuclease is a non-specific DNA-cleaving enzyme that requires dimerization to function. When fused to the TALE DNA-binding domain, FokI becomes targeted to specific DNA sequences. The FokI domains from two TALENs bind adjacent sites on the DNA and dimerize, causing a double strand break at the targeted site.

  This break is then repaired by the cell's natural repair mechanisms, leading to gene disruption, insertion, or correction (Christian *et al.*, 2010).
- **Linker sequence**: The linker sequence connects the TALE DNA-binding domain to the FokI nuclease. This flexible region ensures that the FokI domains are properly positioned to induce the double-strand break when the TALEs are bound to their target sites (Cermak *et al.,* 2011).

These components work together to provide a powerful and precise tool for genome editing, enabling researchers to make targeted modifications to animal genomes for various applications, including functional genomics, disease modelling, and therapeutic development (Joung & Sander, 2013).

Mechanism: (TALEN)

1. TALEN design: A TALEN is designed to target a specific sequence in the animal's genome. (Miller *et al.,* 2011)
2. DNA binding: The TALEN binds to the targeted sequence in the genome. (Sung *et al.,* 2013)
3. FokI activation: The TALEN activates the FokI endonuclease, which cuts the DNA. (Miller *et al.*, 2011)

4. Double-strand break: The FokI endonuclease cuts the DNA at the targeted sequence, creating a double-strand break. (Sung *et al.,* 2013)
5. Repair machinery activation: The cell's repair machinery is activated to repair the break. (Miller *et al.,* 2011)

Zinc Finger Nucleases (ZNF):

Zinc Finger Nucleases (ZFNs) are a type of gene editing technology that use engineered proteins to induce targeted double-strand breaks in DNA. ZFNs combine the DNA-binding capabilities of zinc finger proteins with the DNA-cleaving ability of the FokI nuclease. This fusion allows ZFNs to precisely target specific DNA sequences and introduce breaks, which the cell's repair mechanisms then process, enabling the insertion, deletion, or modification of genetic material.

ZFNs have been used effectively in various organisms, including animals, to study gene function, create genetically modified animals, and develop potential therapies for genetic diseases (Sander & Joung, 2014).

The concept of using zinc finger proteins for DNA targeting originated from studies of natural zinc finger proteins, which are transcription factors that bind specific DNA sequences. Zinc fingers are characterized by the presence of zinc ions coordinating with cysteine and histidine residues in the protein, forming a finger-like structure that interacts with DNA. Researchers began engineering these natural DNA-binding proteins to recognize new DNA sequences by modifying their finger domains. By fusing these engineered zinc finger proteins with the FokI nuclease domain, scientists developed ZFNs capable of targeting specific sequences in the genome. This development was a major step forward in the field of genome editing (Miller *et al.,* 2011; Kim *et al.,* 2010).

Components of the ZNF system for gene editing in animals

The ZFN gene editing system consists of several key components:

- **Zinc Finger Proteins (ZFPs)**: Zinc finger proteins are composed of multiple zinc finger domains, each of which binds to a specific DNA triplet. By designing a combination of zinc finger domains, researchers can create proteins that target particular DNA sequences. These proteins are engineered to recognize and bind to specific regions of the genome (Pabo *et al.,* 2001).
- **FokI nuclease domain**: The FokI nuclease is an enzyme that introduces double strand breaks in DNA. For ZFN applications, the FokI domain is used in its inactive form and requires dimerization to become active. When two ZFNs bind adjacent DNA sequences, the FokI domains dimerize and induce a double strand break at the target site (Urnov *et al.,* 2010).

- **Linker sequence**: The linker sequence connects the zinc finger domains to the FokI nuclease. This flexible region ensures proper positioning of the FokI domains for effective dimerization and DNA cleavage when the zinc finger proteins are bound to their target sequences (Smith *et al.,* 2000).

These components work together to provide a highly specific and versatile tool for genome editing, enabling targeted modifications to animal genomes for research and therapeutic purposes (Sander & Joung, 2014).

Mechanism: (ZNF)

1. ZFN design: A ZFN is designed to target a specific sequence in the animal's genome. (Urnov *et al.,* 2010)
2. DNA binding: The ZFN binds to the targeted sequence in the genome. (Meyer *et al.,* 2012)
3. FokI activation: The ZFN activates the FokI endonuclease, which cuts the DNA. (Urnov *et al.,* 2010)
4. Double-strand break: The FokI endonuclease cuts the DNA at the targeted sequence, creating a double-strand break. (Meyer *et al.*, 2012)
5. Repair machinery activation: The cell's repair machinery is activated to repair the break. (Urnov *et al.,* 2010)

Genome modification by CRISPER/Cas9, TALEN AND ZNF:

Genome modification of animals using ZFN, TALEN, and CRISPR/Cas9 systems has advanced significantly, employing various techniques for precise gene editing. Each method has unique applications depending on the stage of development and type of organism.

Cell manipulation: Cell manipulation techniques enable the introduction of gene editing tools into cells or embryos, facilitating precise modifications in animal genomes. For all three systems— Transcription Activator-Like Effector Nucleases (TALENs), Zinc Finger Nucleases (ZFNs) and CRISPR/Cas9—techniques such as microinjection, electroporation, and somatic cell nuclear transfer (SCNT) are commonly used (Mali *et al.,* 2013; Christian *et al.,* 2010; Urnov *et al.,* 2005).

Manipulation of zygote: The introduction of gene editing components into zygotes is a powerful method for creating genetically modified animals from the earliest developmental stages. For CRISPR/Cas9, TALENs, and ZFNs, microinjection into zygotes allows for the creation of knockout or knock-in models with high efficiency (Mali *et al.,* 2013; Christian *et al.,* 2010; Urnov *et al.,* 2005). This technique ensures that the genetic modification is present in all cells of the developing organism, leading to heritable changes.

Microinjection: Microinjection is a widely used technique for delivering gene editing components directly into the pronucleus of fertilized eggs. For CRISPR/Cas9, TALENs, and

ZFNs, this method involves injecting either the nucleases or their mRNA directly into the embryos (Wang *et al.*, 2013; Doyon *et al.*, 2008; Smith *et al.*, 2000). This direct approach allows for precise modifications and is often used in model organisms such as mice and zebrafish.

Electroporation: Electroporation facilitates the delivery of gene editing tools into cells or embryos by applying an electric field to increase membrane permeability. This technique is employed for CRISPR/Cas9, TALENs, and ZFNs to introduce plasmids or nucleic acids encoding the editing components (Neumann *et al.*, 1982; Wang *et al.*, 2013). It is particularly useful for cells in culture or for embryos where direct injection might be challenging.

Somatic Cell Nuclear Transfer (SCNT): SCNT involves transferring the nucleus of a somatic cell into an enucleated egg cell to create cloned animals. Gene editing tools like CRISPR/Cas9, TALENs, and ZFNs can be used to modify somatic cells before nuclear transfer (Hwang *et al.*, 2004; Miller *et al.*, 2011). This approach is advantageous for generating animals with specific genetic modifications through cloning, allowing for the creation of genetically uniform populations with precise edits.

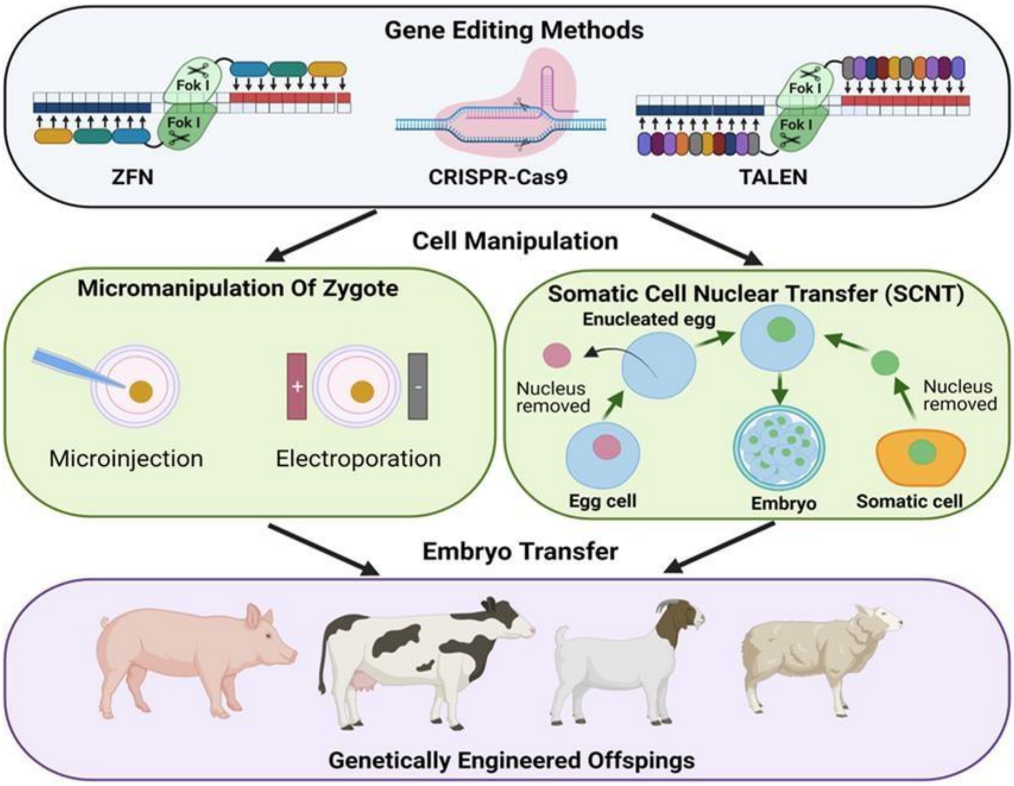

Fig. 3: Genome modification of animals using ZFN, TALEN and CRISPR/Cas9 system.

Source: http://dx.doi.org/10.1007/s11259-022-09967-8

Application in poultry and livestocks

The CRISPR/Cas9 gene editing system has revolutionized the field of animal genetics, enabling precise modifications to the genome of various livestock species including poultry, cattle, goats, sheep, and pigs. Compared to alternative systems like ZFNs and TALENs, CRISPR/Cas9 is more specific, efficient, and widely used for modifying livestock genomes. (Raza, 2022)

Disease resistance

- Pigs: Knocking out the CD163 gene using CRISPR makes pigs completely resistant to Porcine Reproductive and Respiratory Syndrome (PRRS) virus, which costs North American and European pig producers about $6 million per day. (Raza, 2022)
- Cattle: CRISPR can be used to overexpress the NRAMP1 gene in cattle to produce resistance against Mycobacterium bovis infection, which causes economic losses and zoonotic risks.
- Cattle: Zinc finger nucleases can introduce a single amino acid into the bovine CD18 protein, conferring resistance to leukotoxin-mediated cytotoxicity from Pasteurella haemolytica, a major cause of shipping fever in cattle.

Improved performance

- Livestock: Knocking out the myostatin (MSTN) gene results in a double muscling phenotype with hyperplasia and hypertrophy of muscle Fibers, improving meat production. This has been done in Belgian Blue cattle, Texel sheep, and other livestock.
- Cattle, Chickens: Gene editing can produce lines of beef cattle that only produce male offspring, or layer chickens that only produce females, enhancing reproductive performance.
- Pigs: Knocking out the NANOS2 gene in male pigs allows them to produce gametes derived from genetically superior males. (Raza, 2022)

Improved milk quality

- Cattle, Goats: Knocking out the major milk allergen genes β-lactoglobulin in cattle and goats improves milk quality for those with allergies or intolerances.
- Goats: Knocking out β-lactoglobulin and knocking in human lactoferrin in goats reduces milk allergens while adding an important iron absorption and immunity protein.

Animal welfare

- Cattle: Using TALENs, the causative Celtic polled mutation (Pc) was introgressed into the Holstein cattle genome to produce naturally hornless cattle, avoiding painful dehorning procedures. (Raza, 2022)
- Genome editing technologies have significantly transformed the production of transgenic animals within the livestock industry. Previously, gene targeting methods, including gene knockout (KO) and knock-in, posed considerable challenges in livestock breeds due to the absence of germlinetransmittable embryonic stem cells.

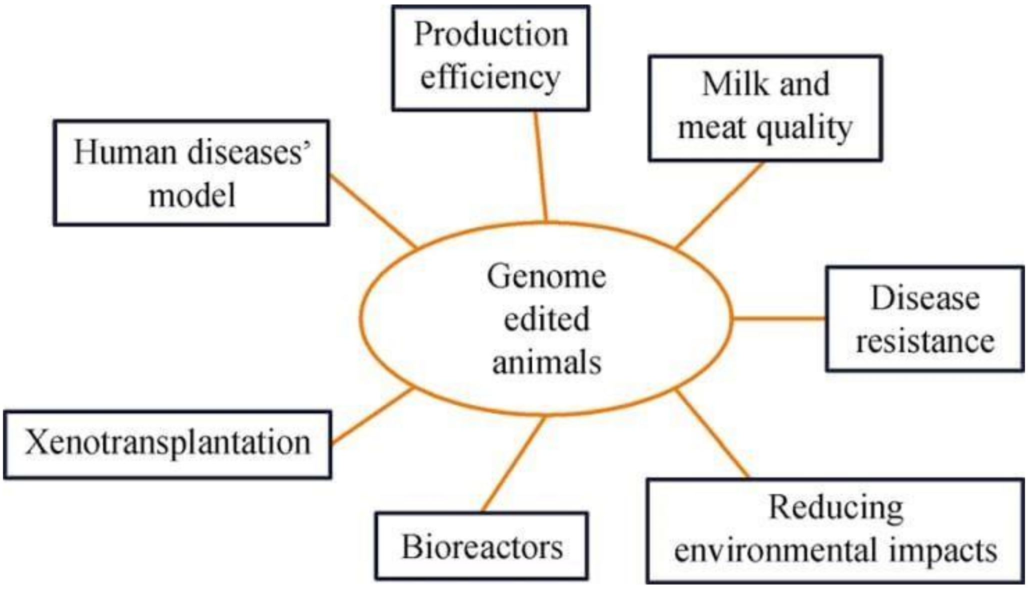

Fig. 4: Applications of genome editing

Source: http://dx.doi.org/10.15302/J-FASE-2016085

Safety and regulatory consideration

The emergence of genome editing technologies has raised significant public concerns regarding ethics, safety, and regulation. Key issues include the frequency of off-target edits resulting from bioengineering and the potential for unintended gene drive generation. These scientific concerns are critical for developing governance and regulatory frameworks for future genome editing products. (JD., 2017)

Off-target edits

Research indicates that the frequency of off-target mutations in CRISPR-Cas9 genome editing, particularly in plant systems, is relatively low. For instance, transgenic soybeans created through traditional Agrobacterium-mediated methods exhibited genomic variations that were significantly less than those seen in radiation-induced soybean mutants and even less than variations among different soybean cultivars.

To build public trust and reduce regulatory uncertainty, it is essential to confirm that the low incidence of off-target effects is consistent across various applications, including those involving Cas9/gRNA-induced mutations. Factors influencing this assessment include the observed increase in editing efficiency over time in cultured soybean embryos, suggesting that continuous expression of Cas9 during development could lead to dose-dependent effects, potentially raising off-target mutation rates. Addressing these concerns will require comprehensive genome analyses of CRISPR-edited plant lines, alongside advancements in computational tools and experimental methodologies to ensure minimal unintended effects at similar genomic sites. (JD., 2017)

Gene drives

The potential creation of gene drives presents another significant concern in genome editing, particularly regarding crop safety and food security. Gene drives enable biased inheritance, increasing the likelihood that offspring will inherit specific genetic traits. While natural gene drives are kept in check by various biological and environmental factors, the design of CRISPR-Cas9 systems can facilitate the creation of highly efficient gene drives when the Cas9 gene and gRNA are incorporated into the same construct with homologous flanking arms targeting embryos or germ cells. (JD., 2017)

This capability necessitates careful research under controlled conditions until the implications of gene drives are better understood. Whether created intentionally or inadvertently gene drives result in monoallelic gene expression, as CRISPR-Cas9 continues to edit genes in subsequent generations. (JD., 2017)

Currently, there are 18 gene-editing-based therapeutics in clinical trials globally. Most of these trials (61%) utilize zinc-finger nucleases (ZFNs), with 11 studies in total, six of which are organized by Sangamo Bioscience. Among the ZFN trials, three are Phase I/II studies aimed at targeting the CCR5 gene to combat HIV infection. The remaining eight ZFN trials are Phase I studies that vary in target genes, diseases, and delivery methods. (Shim, 2017)

In the realm of transcription activator-like effector nucleases (TALENs), there are three ongoing Phase I trials focusing on multiplex gene editing of TCRa (T cell receptor alpha) and CD52 to enhance the effectiveness of chimeric antigen receptor (CAR) T cell therapies targeting CD19 (specifically UCART19, which is licensed to Servier). The CRISPR/Cas9 system has also been recently employed in clinical trials in China, targeting the PDCD1 gene as a treatment for various cancers. (Shim, 2017)

The delivery strategies for gene editing in these clinical trials fall into three categories: viral vectors (6 studies), electroporation (6 studies), and naked plasmids (1 study). Specifically, the ex vivo gene-editing strategies aimed at CCR5 to inhibit HIV entry utilize adenoviral vectors (4 studies) or electroporation (3 studies) for T cell transfection. Additionally, electroporation of TALEN mRNA is used in three studies for TCRa/CD52 knockout in CD19-CAR-T trials. While details on the delivery methods for the four ex vivo CRISPR/Cas9 trials are not yet available, it is presumed that viral vectors or electroporation methods are being utilized due to their effectiveness in T cell transfection. (Shim, 2017)

In vivo administration using AAV vectors has been employed for hepatocyte-targeted gene-editing therapies, such as those for haemophilia B and MPS I. A notable candidate, the CCR5-knockout T cell line (SB-728-T), was developed through adenoviral delivery of ZFN. In a Phase I safety study (NCT00842634), 12 HIV-infected patients received a single infusion of 5×10^9 to 10×10^9 CCR5-knockout T cells, which maintained a consistent blood concentration with a mean half-life of 48 weeks, compared to a loss rate of 7.25 cells per day for unmodified T cells. Preliminary results from a Phase II trial indicated that the adenoviral delivery strategy also promoted additional CD8-mediated immune stimulation, suggesting an adjuvant-like effect from the adenovirus used in the gene editing. (Shim, 2017)

Regulatory perspectives

As the number of studies exploring gene-editing-based therapeutics increases, addressing regulatory concerns that may impact clinical applications becomes essential. While it is challenging to outline all potential regulatory issues, the main concerns revolve around safety, efficacy, and quality control. The genetic materials used to deliver gene-editing nucleases are similar to those used in conventional ex vivo/in vivo gene therapies, allowing for quality control and efficacy evaluations to be aligned with existing gene therapy guidelines.

The guidelines issued by the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) provide a framework for gene-editing therapeutics, but the introduction of novel gene-editing mechanisms using exogenous nucleases raises new safety concerns. (Duensing, 2018)

Gene-editing therapeutics add complexity to delivery methods and systems, with varying risk levels depending on the delivery mode. Generally, direct in vivo delivery carries higher risks compared to ex vivo delivery of gene-edited cell therapeutics. The type of delivery system itself can also influence risk levels; for example, viral vectors may pose greater safety concerns compared to non-viral vectors or electroporation methods. Due to the elevated risks associated with in vivo gene editing at this developmental stage, most gene-editing-based therapeutics in clinical trials currently employ ex vivo strategies. This review will focus primarily on the ex vivo delivery form of gene-editing therapeutics. (Duensing, 2018)

The implications of regulatory alternatives for genome editing technologies are multifaceted, impacting social perception, international trade, local innovation, and the competitiveness of agroindustrial chains. Jeffrey Wolt highlights the National Academies of Sciences, Engineering, and Medicine (US-NASEM) report, which emphasizes the need to modernize the biotechnology regulatory system in the U.S. This modernization aims to clarify agency roles, formulate long-term strategies for risk assessment of future biotechnology products, and support innovation while ensuring public health and environmental protection. (Duensing, 2018)

In 2015, a White House Memorandum initiated efforts to update the Coordinated Framework for Biotechnology, focusing on enhancing transparency, predictability, and reducing regulatory burdens. This included commissioning an independent analysis of the future landscape of biotechnology products to address potential new risks and risk assessment frameworks expected to emerge over the next 5–10 years. The report produced by the US-NASEM committee acknowledges the rapid growth of the bioeconomy and the necessity for the regulatory system to adapt accordingly.

While the report does not specifically address genome-edited crops, it recognizes them as an existing reality within the U.S. regulatory framework. This perspective suggests that how risk and regulatory considerations for genome-edited crops are managed will significantly influence the entry of innovative biotechnologies into the marketplace. (Duensing, 2018)

Regulators and policymakers are becoming more familiar with the technical aspects of genome editing, leading to global debates on appropriate regulatory measures. Issues such as "productbased" versus "process-based" regulation and product detectability have emerged but may not significantly contribute to decision-making. It is crucial that these technical debates aid in interpreting and modifying the regulatory frameworks of individual countries.

Many nations engaged in international trade have begun to establish or refine their regulatory criteria. At this stage, the primary challenges to creating a cohesive and globally harmonized regulatory environment are more social than technical. Therefore, it is essential to assess the implications of regulatory alternatives on social perception, international trade, local innovation, and the competitiveness of agro-industrial chains. (Duensing, 2018)

Future directions

Gene editing for animal improvement and disease resistance are revolutionizing agriculture by enhancing precision and efficiency. Advances in technologies like CRISPR-Cas9 are enabling more targeted modifications, allowing for the enhancement of multiple traits simultaneously, such as growth rate, feed efficiency, and production quality. This precision editing is pivotal in developing animals with natural resistance to major diseases, reducing reliance on antibiotics and vaccines, and bolstering overall animal health. Additionally, gene editing is being used to adapt animals to specific environmental conditions, promoting sustainability in diverse farming systems by reducing the resources needed for production.

As these technologies advance, ethical considerations and regulatory frameworks are playing a crucial role in guiding responsible use, ensuring that animal welfare remains a priority. New biotechnological frontiers, such as gene drives and epigenetic modifications, are offering innovative approaches to population control and dynamic trait management. Integration with artificial intelligence and emerging gene-editing technologies like base and prime editing is further enhancing the precision and outcomes of genetic modifications. Together, these advancements are creating a more sustainable and resilient agricultural system capable of meeting global challenges in food security and animal health. (Liu Z, 2022)

Conclusion:

In conclusion, gene editing technologies such as CRISPR, TALEN, and ZFN hold transformative potential for improving animal traits and enhancing disease resistance in livestock. These advancements can lead to significant gains in productivity, health, and welfare across various species, from increased growth rates and improved product quality to enhanced resistance against viral, bacterial, and parasitic diseases. While the applications in livestock farming are promising, it is crucial to consider safety, regulatory frameworks, and ethical implications, especially regarding off-target effects and germline editing. As gene editing continues to evolve, its integration with other biotechnologies and careful consideration of public acceptance will be key to realizing its full potential in animal agriculture and conservation efforts.

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NUTRITIONAL STRATEGIES FOR ENHANCING GROWTH AND HEALTH IN CULTURED FISH

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Abstract:

In aquaculture, optimizing nutritional strategies is essential for enhancing growth and health in cultured fish. This chapter discusses the fundamental role of macronutrients and micronutrients, their specific requirements at various life stages, and the importance of balanced diets. The chapter also explores functional feeds, including probiotics and prebiotics, that support immune function and disease resistance. Practical feed formulation and its impact on fish physiology are examined, along with sustainable practices for minimizing environmental impact. Through a review of current research and case studies, this chapter provides actionable insights for improving the overall health and productivity of farmed fish.

Keywords: Macronutrients, Micronutrients, Functional Feed, Nutraceuticals, Growth

Introduction:

Aquaculture has become a critical component of global food production, providing nearly half of all seafood consumed worldwide. As the industry expands, ensuring the optimal health and growth of cultured fish through effective nutritional strategies is vital. Proper nutrition not only promotes growth but also enhances disease resistance, improves feed efficiency, and reduces environmental impact. This chapter aims to provide a comprehensive overview of the nutritional strategies used in aquaculture to enhance the growth and health of cultured fish. By understanding the specific dietary needs of fish and incorporating innovative feeding practices, aquaculture producers can achieve better growth rates, improve fish health, and contribute to the sustainability of the industry.

Macronutrients and their role in fish growth

1. Proteins

Proteins are the primary building blocks for growth and tissue repair in fish. They provide essential amino acids that are necessary for various physiological functions, including enzyme and hormone production. Fish require high levels of protein in their diets, particularly during early life stages when growth is rapid. Sources of protein in aquafeeds include fish meal, soybean meal, and alternative proteins like insect meal and algae.

Optimizing protein levels in feed formulations is crucial to support efficient growth while minimizing nitrogen waste, which can contribute to water pollution.

2. Lipids

Lipids are a vital source of energy for fish and play a significant role in cellular function, including maintaining cell membrane integrity and supporting the absorption of fat-soluble vitamins. Essential fatty acids, such as omega-3 and omega-6, are important for normal growth, reproductive health, and immune function in fish. Fish oils and plant oils are common lipid sources in aquafeeds. Balancing the lipid content in the diet is necessary to provide sufficient energy while preventing excessive fat deposition, which can lead to metabolic disorders.

3. Carbohydrates

Although fish are generally less efficient at utilizing carbohydrates compared to terrestrial animals, carbohydrates can serve as an energy source, sparing protein for growth. The inclusion of carbohydrates in fish diets must be carefully managed, as excessive levels can lead to reduced feed efficiency and adverse health effects. Starches and simple sugars are the primary carbohydrate sources in aquafeeds, and their digestibility varies depending on the fish species and life stage

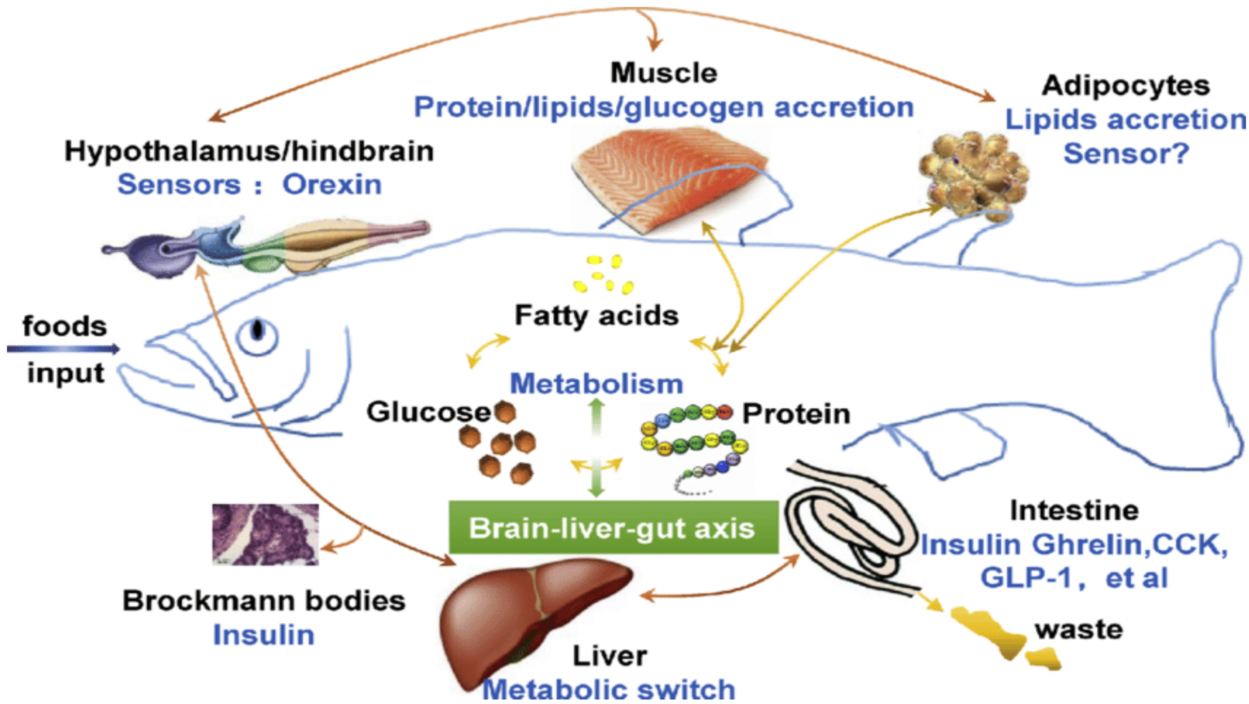
Micronutrients and fish health

1. **Vitamins**

Vitamins are organic compounds essential for various metabolic processes. Fat-soluble vitamins, such as A, D, E, and K, are crucial for growth, bone health, and immune function. Vitamin C, a water-soluble vitamin, plays a critical role in collagen formation and wound healing. Deficiencies in essential vitamins can lead to various health issues, including skeletal deformities, impaired growth, and increased susceptibility to diseases. Ensuring adequate vitamin levels in fish diets is important for maintaining overall health and preventing deficiency-related conditions.

2. **Minerals**

Minerals such as calcium, phosphorus, magnesium, and trace elements like zinc and selenium are necessary for skeletal development, osmoregulation, and enzyme function in fish. Imbalances or deficiencies in minerals can result in poor growth, weakened bones, and impaired immune responses. While some minerals can be absorbed from the water, others must be provided through the diet. Careful attention to mineral supplementation in feeds is essential for supporting healthy growth and physiological functions in fish.



Functional feeds and nutraceuticals

Functional feeds are formulated to provide additional benefits beyond basic nutrition, often including ingredients that support the immune system, enhance stress resistance, and promote overall health. Probiotics and prebiotics are among the most studied functional feed components. Probiotics are live microorganisms that confer health benefits by improving gut health and enhancing immune responses. Prebiotics, on the other hand, are non-digestible food components that stimulate the growth of beneficial bacteria in the gut, further supporting digestive health and disease resistance. The use of functional feeds in aquaculture has shown promise in reducing the incidence of diseases, improving growth performance, and enhancing the overall well-being of fish. Incorporating these nutraceuticals into standard feed formulations can be an effective strategy for enhancing the resilience and productivity of cultured fish populations.

Practical feed formulation strategies

Feed formulation is a critical aspect of aquaculture nutrition, involving the precise combination of ingredients to meet the specific nutritional needs of different fish species at various life stages. The formulation process considers factors such as protein and energy requirements, the digestibility of feed ingredients, and the availability of nutrients. Advances in feed formulation techniques have enabled the development of more efficient feeds that support optimal growth while minimizing waste output. Sustainable feed formulation practices are increasingly important, focusing on reducing the reliance on fish meal and fish oil by incorporating alternative protein and lipid sources. These practices help reduce the environmental impact of aquaculture and contribute to the long-term viability of the industry.

Nutritional management for disease prevention

Proper nutrition is integral to disease prevention in aquaculture. Fish with well-balanced diets are more resilient to environmental stressors and less susceptible to infections. Nutritional management strategies, including the use of functional feeds, can play a crucial role in enhancing the immune system and preventing common diseases in cultured fish. Additionally, maintaining optimal water quality through proper feeding practices can further reduce the risk of disease outbreaks and support overall fish health.

Conclusion:

Nutritional strategies are central to the success of aquaculture, directly influencing the growth, health, and sustainability of cultured fish populations. By understanding the specific nutritional requirements of fish and incorporating innovative feeding practices, aquaculture producers can optimize growth performance and improve the overall health of their stock. The continued development of functional feeds and sustainable feed formulations will be key to meeting the challenges of modern aquaculture, ensuring that the industry remains viable and productive in the face of growing global demand

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FROM TRADITIONAL TO CUTTING-EDGE: GENE IMPROVEMENT IN LIVESTOCK

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Abstract:

Gene improvement in livestock has evolved from traditional selective breeding, starting with animal domestication around 10,000 to 8,000 BCE, to advanced biotechnological techniques today. Breeding techniques evolved during the course of the Middle Ages, with notable scientific breakthroughs in the 18th and 19th centuries and the development of groundbreaking genetic technology in the 20th and 21st centuries. Contemporary methods such as marker-assisted selection and genomic selection (MAS), as well as gene editing, are currently essential for improving livestock characteristics like production, illness resistance as well as flexibility. Conventional techniques like as artificial insemination, crossbreeding, and natural and artificial selection are still important. SNP markers are used in genomic selection to provide more precise breeding values, and QTL mapping and genomic tools are used to discover genes associated with desired traits. Effective breeding programs now integrate these modern techniques with traditional methods to achieve targeted improvements, driving significant advancements in livestock breeding and agricultural productivity.

Introduction:

Genetic enhancement, or gene improvement, is the process of applying several strategies to improve desired qualities in livestock, such as disease resistance, production, and adaptability. This process has evolved from traditional methods like selective breeding to advanced technologies such as genetic engineering and marker-assisted selection (Zenger *et al.*, 2019).

Historical development

- **Ancient practices:** Livestock breeding began with the domestication of animals around 10,000 to 8,000 BCE, where early breeders selected animals based on observable traits like size and temperament (Zeder, 2008)
- **The Middle Ages and the renaissance periods:** It saw the emergence of systematic selective breeding, which was accompanied by the widespread use of recorded procedures and breed standards (Hoffman, 1999; Davis, 2003).

- **18th and 19th Centuries:** The scientific approach to breeding began, pioneered by figures like Robert Bakewell. Darwin's theory of evolution and the early development of quantitative genetics also influenced breeding practices (Lush, 1937; Darwin, 1859; Fisher, 1918).
- **20th century:** The 20th century saw significant developments in breeding technologies and genetics, including the creation of artificial insemination (AI) and embryo transfer methods, as well as the discovery of DNA structure by Watson and Crick (Watson & Crick, 1953; Foote, 2002).
- **21st Century:** Biotechnology and genomics have further revolutionized livestock breeding, with techniques like marker-assisted selection (MAS) and gene editing through CRISPR being at the forefront of modern genetic improvement (Ribaut & Ragot, 2007; Hsu *et al.,* 2014).

Modern techniques and ethical considerations

- **Assisted Reproductive Technologies (ART) and Genomic Selection (GS):** These contemporary methods enable more accurate selection and utilization of genetically superior parents to expedite genetic gain (Mueller & Van Eenennaam, 2022).
- **Artificial insemination:** This has facilitated the widespread dissemination of superior genetics, improving the overall efficiency of livestock production (Foote, 2002).
- **Ethical and environmental considerations:** The advancements in genetic technologies also bring about ethical and environmental concerns that must be managed to ensure sustainable and responsible practices in agriculture (Van Eenennaam, 2019).

Breeding techniques

In the age of genomic selection, traditional animal breeding methods continue to be crucial for selecting and enhancing animals. Animal breeding focuses on selectively mating domestic animals to boost desirable and heritable traits in future generations. An animal’s overall performance is largely determined by its genetic potential inherited from its parents, as well as environmental factors such as nutrition, health, management, and other factors.

A) Traditional breeding methods:

Selection is a key process in traditional animal breeding, where the best animals are chosen as parents to produce the next generation. By boosting the frequency of advantageous genes and decreasing the frequency of undesirable ones, the intention is to improve desirable features. Multiple qualities are emphasized in this human-controlled procedure to produce elite breeding stock, which boosts production. An animal's performance is influenced by its genetic potential as well as environmental elements including care, diet, and health. (Thakur, 2022).

Types of selection

Natural selection and artificial selection are the two categories under which selection falls. The techniques used in artificial selection include the Tandem method, Independent Culling Level, Selection Index, and index selection.

Natural selection: The "survival of the fittest," or the survival and reproduction of animals most adapted to their surroundings, is the driving force behind natural selection. This process is influenced by factors such as variations in mortality rates (especially in early life), differences in the length and level of sexual activity, and fertility rates. Through mutations and genetic recombination, less successful genetic combinations are eliminated, while successful adaptations thrive.

Artificial selection:

- **Automatic selection:** Natural reproduction with minimal human intervention.
- **Deliberate selection:** Intentional breeding based on specific desirable traits.
- **Replacement selection and culling:** Selecting new breeding animals to replace those removed from the population.

Breeding Methods:

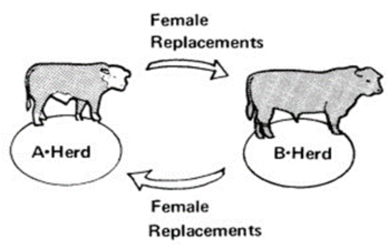
- **Tandem selection:** Focuses on improving one trait at a time. Efficiency is limited by the genetic correlation between traits (noted for its simplicity but often slow and discouraging) (Singh, 1936).
- **Independent culling levels:** Evaluates animals on multiple traits, each needing to meet a minimum standard. Can be too rigid, potentially rejecting genetically superior animals (Singh, 1936).
- **Selection index method:** Weighted by genetic ties and economic value, a total score is used to assess animals across many attributes. It is the most effective method for handling multiple traits but requires accurate and updated data (Singh, 1936).

Crossbreeding

Crossbreeding is a strategy where animals from different breeds are mated to combine their desirable traits, producing hybrid offspring that often outperform their parents. The primary goal is to harness hybrid vigor (heterosis), where crossbred animals exhibit superior qualities such as growth rate, fertility, and disease resistance compared to purebred parents.

Types of crossbreeding:

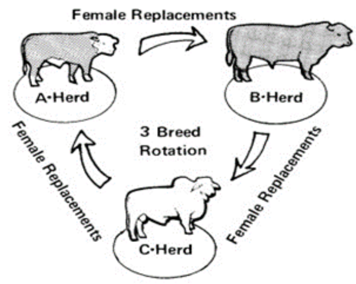
1. **Criss-cross or two-breed cross:**



This method involves mating individuals from two pure breeds to produce F1 hybrids. These hybrids are heterozygous, meaning that they have better characteristics like growth rate and reproductive efficiency. Nonetheless, the first generation exhibits the strongest hybrid vigor, frequently necessitating further crossbreeding techniques to sustain performance.

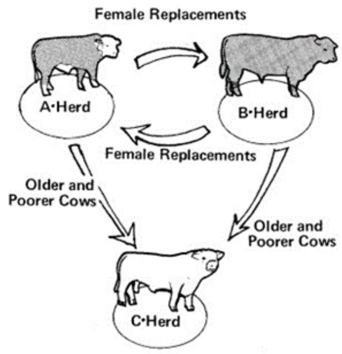
2. **Rotational cross:**

In rotational crossbreeding, two or more breeds are used in a planned rotation. Females of one breed are mated with males from another, and this pattern continues in subsequent generations. This method maintains continuous heterosis and high performance by balancing traits from multiple breeds. However, it requires careful management and record-keeping, as trait expression can fluctuate.



3. **Terminal cross:**

In terminal crossbreeding, crossbred females are mated with males selected for market traits, producing offspring solely for market use. This method maximizes hybrid vigor for traits like meat quality or growth rate. It requires a constant supply of replacement females, which adds complexity and cost to the breeding program. (Quintana *et al.*, 1983).



1. **Artificial Insemination (AI):**

Artificial insemination is a widely used reproductive technology in animal husbandry aimed at enhancing the genetic quality of livestock. It involves collecting semen from a genetically superior male and manually depositing it into the reproductive tract of a female at the optimal time for fertilization.

Process of Artificial Insemination:

Process:

- **i** **Bull fertility assessment:** Includes physical examination and breeding soundness evaluation.
- **ii** **Semen collection methods:** Common methods are artificial vagina (AV), electro-ejaculation, and transrectal massage.
- **iii** **Semen evaluation:** Includes macroscopic tests for volume and color, and microscopic tests for motility and morphology.
- **iv** **Semen processing and preservation:** Semen is diluted and preserved via cooling or cryopreservation.

2. Embryo transfer

Using an embryo donor female and one or more recipient females, embryo transfer (ET) is a reproductive method. ET is frequently employed in cattle breeding to increase genetically superior cows' capacity for reproduction.

Steps Involved in Embryo Transfer:

1. **Selection of donor cow:** Choose a genetically superior cow with a good reproductive history and no defects.
2. **Superovulation:** Administer FSH to induce multiple egg releases during one estrus cycle.
3. **Insemination:** Fertilize the superovulated cow multiple times with high-quality semen.
4. **Flushing embryos:** Collect embryos from the uterus by flushing with a special medium seven days after insemination.
5. **Embryo evaluation:** Assess and select high-quality embryos based on their development
6. **Transfer to recipients:** Transfer selected embryos to synchronized recipient cows for implantation. (Curtis, 2015).

3. Cloning

Animal cloning is a technique that produces genetically identical copies of an animal. This technology is primarily used in agriculture to replicate elite animals with desirable traits, such as high milk production or rapid growth.

Cloning process

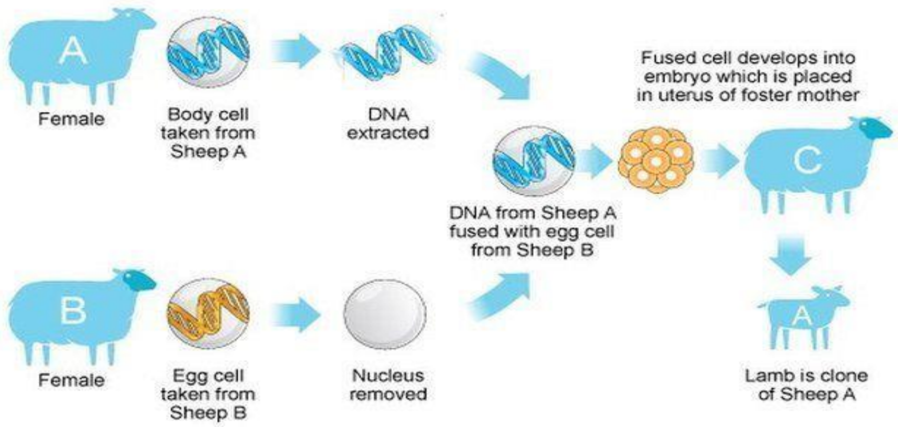
The most common method for cloning animals is Somatic Cell Nuclear Transfer (SCNT). This involves the following steps:

1. **Cell collection:** From the donor animal, a somatic (a non-reproductive cell) cell is taken.
2. **Enucleation:** An egg cell is taken from another animal, and its nucleus is removed, creating an enucleated egg.
3. **Fusion:** The donor cell is fused with the enucleated egg using an electrical pulse.
4. **Embryo development:** The fused cell begins to divide and develop into an embryo.
5. **Transfer to surrogate:** The embryo is implanted into a surrogate mother, where it continues to develop until birth.

Genomic selection

Traditional Animal Breeding (TAB) primarily focuses on selecting animals based on their performance records and physical traits. This method estimates breeding values (EBV) from an animal's and its family's phenotypes (Ventura *et al.*, 2016). Despite its historical effectiveness, TAB often results in lower reliability and less optimal production outcomes due to its reliance on phenotypic data alone.

Animal breeding has undergone a revolution thanks to genomic selection, which has been made possible by advances in quantitative genetics. Compared to conventional approaches, our strategy predicts genetic merit more precisely by using dense panels of single nucleotide polymorphism (SNP) markers (Deng *et al.*, 2016; de Koning, 2016). Genomic selection integrates both genotypic and phenotypic data from a reference population to develop predictive equations for estimating breeding values. This approach allows for improved selection accuracy, particularly for young candidates, by enhancing the estimation of breeding values through a larger reference population.



Marker-Assisted Selection (MAS)

It leverages genetic markers associated with desirable traits to improve breeding programs, particularly for traits with straightforward genetic background. MAS accelerates the selection process by predicting traits that may not be easily observed phenotypically but is less effective for complex traits due to its reliance on a limited number of genetic markers. Genomic Selection (GS) enhances MAS by analyzing single nucleotide polymorphisms (SNPs) across the entire genome to estimate breeding values more accurately. GS uses comprehensive data from both phenotypes and genotypes within a reference population to create predictive equations for genomic estimated breeding values (GEBV). This approach improves accuracy by integrating multiple genetic markers, considering factors like trait heritability, the reference population size, and the strength of marker-trait associations. As a result, GS has led to significant advances in livestock productivity and genetic progress by providing more precise and efficient selection tools.

Quantitative Trait Loci (QTL)

A chromosomal locus refers to a particular site on a chromosome where a gene or group of genes that affect a quantitative trait resides. These traits, also known as polygenic features, are controlled by multiple genes and loci. In 1975, Gelderman originally put out the concept of quantitative trait loci, or QTLs.

QTL mapping techniques

1. **Linkage analysis:** Traditionally used to associate genetic markers with traits by examining inheritance patterns in pedigreed populations. This method identifies QTL by tracking the co-segregation of markers with traits across generations.
2. **Genome-Wide Association Studies (GWAS):** These studies scan the entire genome using high-density single nucleotide polymorphisms (SNPs). GWAS provides a thorough understanding of the genetic architecture of phenotypes by correlating SNP changes with phenotypic data from large populations (Mackay *et al.,* 2012).
3. **High-density SNP genotyping:** Advances in SNP genotyping technology allow for more precise QTL mapping by examining fine-scale genetic variations. This enhances the resolution of QTL mapping and facilitates more accurate selection (König *et al.,* 2010).

Despite its advancements, QTL mapping faces challenges, such as identifying QTL for traits with low heritability or complex genetic interactions. The cost and complexity of high density genotyping and data analysis can also be limiting factors. Future research is expected to integrate QTL mapping with other genomic tools, like whole-genome sequencing and functional genomics, to further refine trait genetics and improve breeding outcomes (Goddard & Hayes, 2009).

Traits of interest

Traits of interest in animal breeding refer to genetically influenced characteristics selected to enhance productivity, efficiency, and sustainability in agriculture. These characteristics were selected for their economic worth, effect on animal welfare, and ability to support sustainable agricultural methods.

1. **Genetic traits**

- **Heritability:** Reflects the extent to which genetic factors contribute to trait variation within a population. The probability of inheriting features from parents to children rises with high heritability.
- **Genetic improvement:** Techniques like selective breeding and genetic modification aim to enhance traits such as growth rate, disease resistance, and milk production.

2. **Productivity traits**

- **Milk production:** Involves the quantity and quality of milk, including factors like yield, fat content, and protein levels, crucial for dairy farming.
- **Growth rate:** The speed at which animals reach market weight, important for meat production, and involves feed conversion efficiency.

3. **Reproductive traits**

- **Fertility:** Encompasses reproductive efficiency traits like conception rates and calving intervals.
- **Litter size:** Refers to the number of offspring in one birthing event, with larger litter sizes being advantageous in breeding programs.

4. **Health traits**

- **Disease resistance:** The ability to resist or recover from diseases, influenced by genetic and immune factors.
- **Longevity:** An animal's lifetime, which is crucial for breeding and welfare, is impacted by both genetics and environment.

5. **Behavioural traits**

- **Temperament:** Behavioural traits like docility or aggression, affecting interactions with humans and other animals.
- **Trainability:** The ease with which animals learn new behaviours, crucial for working and companion animals.

6. Adaptation traits

- **Climate adaptation:** Traits that enable animals to thrive in various environments, such as heat tolerance.
- **Nutritional adaptation:** How animals adjust feeding behaviour based on food availability and quality.

7. Conservation traits

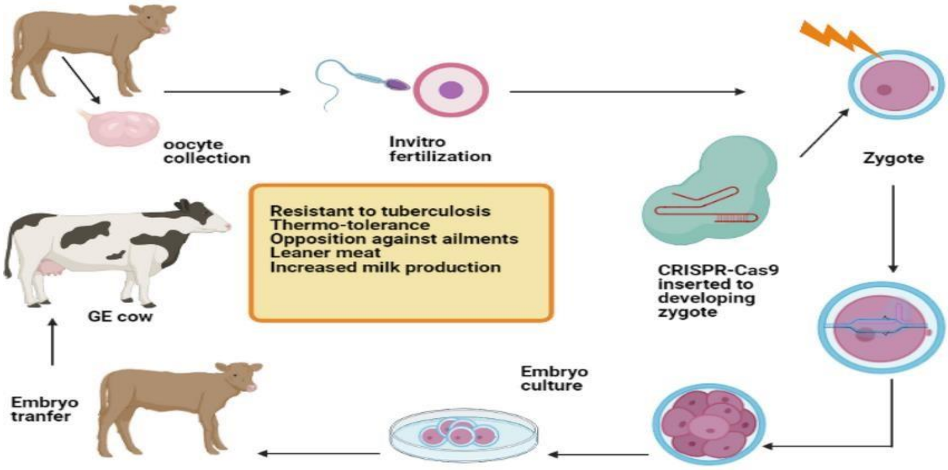
- **Endangered species traits:** Characteristics vital for the survival of endangered species, including genetic diversity.
- **Genetic diversity:** The range of genetic variations within a species, essential for resilience and survival.

Future trends in genetic improvement

1. **CRISPR and gene editing technologies:**

- **Precision editing:** CRISPR-Cas9 enables accurate genome editing in livestock to improve features like growth rates and disease resistance while minimizing unwanted genetic effects (Khatryn *et al.,* 2020).
- **Disease resistance:** Gene editing can improve resistance to specific diseases, potentially reducing the need for antibiotics and improving overall health (Bartley *et al.,* 2020).
- **Improved quality traits:** Editing genes related to meat quality, milk yield, or wool characteristics can lead to better product quality and increased profitability (Bennett *et al.,* 2019).

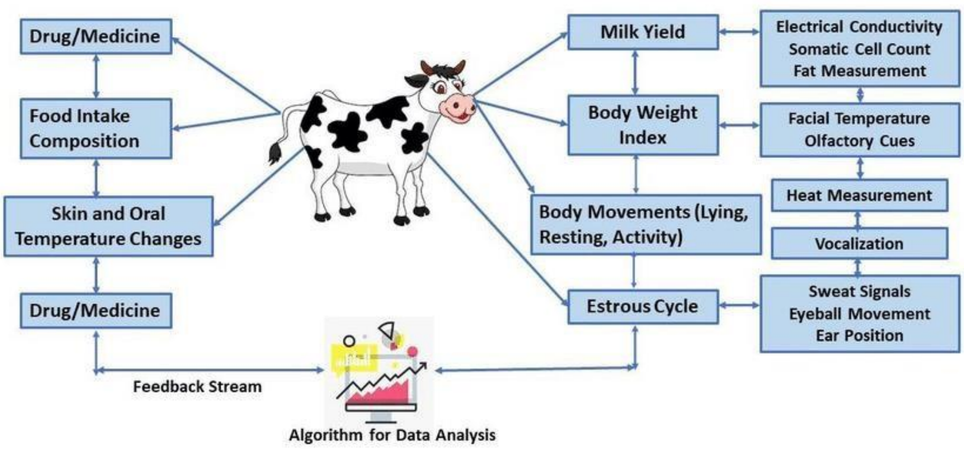
- **Ethical and regulatory considerations:** Concerns about animal welfare and longterm ecological effects are just a couple of the ethical and regulatory challenges that gene editing brings up (Cohen, 2018).



2. **Big data and bioinformatics:**

- **Genomic selection:** Big data tools are used for genomic selection, predicting and selecting animals with superior traits based on extensive genetic data (Goddard & Hayes, 2009).
- **Data integration:** Integrating genomic, phenotypic, and environmental data helps in optimizing breeding programs and understanding complex traits (Hickey *et al.*, 2017).
- **Predictive models:** Machine learning techniques develop predictive models for trait performance, enabling targeted breeding strategies (Raleigh *et al.*, 2018). These trends are driving advancements in livestock genetics, focusing on enhancing productivity and sustainability.

Overall, the combination of gene editing technologies and big data analytics is likely to drive significant advancements in livestock genetics, leading to more efficient, sustainable, and profitable animal production systems.



Case studies

Here are some successful case studies of genetic improvement in various livestock species:

1) Cattle

Holstein dairy cattle

- Case Study: The Holstein breed has undergone significant genetic improvement in milk production through selective breeding. By focusing on traits such as milk yield, fat content, and overall health, genetic selection has led to increased production efficiency and better milk quality.
- Outcome: Modern Holsteins produce approximately 22,000-24,000 pounds of milk per year, significantly higher than historical averages. Advances in genomics have further refined selection strategies, improving reproductive efficiency and disease resistance.

2) Pigs

Pig breeds for increased lean meat

- Case Study: In the pig industry, the focus has been on improving lean meat yield and growth rates. Breeds such as the Pietrain have been genetically selected for higher muscle mass and reduced fat content.
- Outcome: Genetic improvements have led to pigs with higher feed conversion ratios and faster growth rates. The introduction of markers for traits like muscle development has resulted in more efficient pork production with better meat quality.

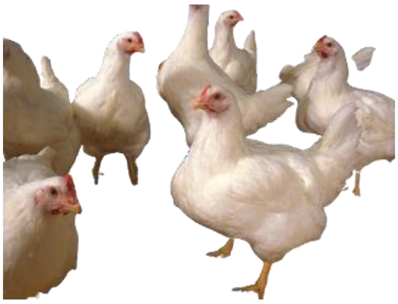
3) Sheep

Merino sheep for wool quality

- Case Study: The Merino breed has undergone selective breeding to improve the length, diameter, and overall fleece output of its wool. The goal of genetic advancements has been to increase fleece yield and decrease wool flaws.
- Outcome: Modern Merinos are extremely valuable in the wool business because they produce high-quality wool with finer fiber diameters and higher yields. Entire flock health and enhanced disease resistance have also been enhanced via genetic selection.

4) Poultry

Broiler chickens for growth rate



- Case Study: Broiler chickens have been genetically improved to enhance growth rates and feed efficiency. Selective breeding has targeted traits such as muscle development, feed conversion efficiency, and resistance to common diseases.
- Outcome: Broiler chickens now reach market weight in a shorter time frame, with better feed conversion ratios. These improvements have led to a reduction in production costs and increased profitability for poultry producers.

These case studies illustrate how genetic improvement through selective breeding and advanced techniques can lead to significant enhancements in livestock productivity, health, and product quality

Conclusion:

Livestock genetic improvement has evolved from traditional selective breeding to advanced technologies like genomic selection and artificial insemination. These innovations have greatly y enhanced productivity, disease resistance, and adaptability. Moving forward, balancing these advancements with ethical and environmental considerations is crucial for sustainable and efficient livestock farming.

Genetic improvement in livestock, driven by advances in QTL mapping, genomic selection, and biotechnologies, has substantially enhanced productivity, health, and efficiency across species. Techniques such as CRISPR and big data analytics are shaping future trends, offering precise trait modifications and personalized breeding strategies. However, challenges like maintaining genetic diversity, ethical concerns, and technological limitations remain. Continued innovation and thoughtful integration of genetic advancements are crucial for sustainable and effective livestock breeding practices.

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A REVIEW OF RESEARCH AND DEVELOPMENT IN THE FIELD OF ANIMAL SCIENCE

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Introduction:

The field of animal science is continuously evolving, driven by advances in technology, genetics and our understanding of animal biology and welfare. Here are some key frontiers in animal science research and development:

1. Genetics and genomics in animal research are developing quickly and are essential to enhancing the welfare, production and health of animals [1]. Here is a deeper look at the main ideas and developments in genetics and genomics today:

1.1. Selection based on genomes

- **Definition:** A breeding strategy that predicts animal performance using genomic data (such as DNA markers) to enable more precise breeding candidate selection.
- **Uses:** Improving characteristics include growth rate, feed efficiency, resistance to illness and reproductive efficiency.
- **Advantages:** Compared to conventional approaches, this method provides more accurate assessments of an animal's genetic potential, which speeds up genetic advancement.

1.2. Gene editing technologies

- **CRISPR-Cas9**: An innovative tool that enables accurate genome editing of animals, including targeted gene insertions, deletions and repairs [2].
- **Uses:** producing animals with desirable features, enhancing disease resistance and producing genetically altered animals for study.

1.3. Whole-Genome Sequencing (WGS)

- **Definition:** This technique involves sequencing an animal's complete genome to detect genetic differences as well as the functional implications of those alterations.
- **Uses:** Determining the genetic origins of illnesses, enhancing breeding programs and identifying genetic markers linked to certain features.
- **Advantages:** Offers a complete comprehension of the genome, resulting in an improved understanding of genetic variety and health [3].

1.4. Proteomics and transcriptomics

- **Transcriptomics:** The investigation of RNA transcripts to learn about the patterns of gene expression in various tissues and situations.
- **Proteomics:** The study of all the proteins that are expressed by a cell, tissue, or organism.
- **Applications:** Finding biomarkers for health and performance, as well as understanding how gene expression varies in response to illnesses, environmental factors, or medical interventions [4].

1.5. Genomic function

- **Definition:** The study of the relationship between a gene's activity and its function in a living organism.
- **Applications:** Investigating the effects of genetic variants on biological processes and phenotypes, as well as creating focused therapies to improve the well-being and productivity of animals.
- **Methods:** RNA interference, gene overexpression and gene knockouts are among them.

1.6. Epigenetics

- **Definition:** The study of variations in gene expression brought on by environmental influences rather than modifications to the underlying DNA sequence.
- **Applications:** Examining how environmental elements such as stress and food affect animal health and gene expression, as well as the possibility of using epigenetic alterations to enhance the quality of offspring [5].

1.7. Population genomics

- **Definition:** The study of genetic variation within and among populations to comprehend genetic diversity, adaptability and evolutionary processes.
- **Uses:** Understanding how animals adapt to various habitats, controlling genetic variety in breeding programs and conservation genetics.

1.8. Databases and genetic resources

- **Genetic databases:** Extensive databases including genetic data, such as trait information, genetic markers and genomic sequences.
- **Uses:** Supporting breeding initiatives, research and the development of genetic testing.

1.9. Personalized medicine and precision livestock farming

- **Personalized medicine:** Tailoring veterinary treatments and interventions to the genetic profile of individual animals.

- **Precision livestock farming:** Integrating genomic data with real-time monitoring to optimize management practices for individual animals based on their genetic potential and health status [6].

1.10. Ethical and regulatory considerations

- **Ethics:** Addressing concerns related to animal welfare, the potential for unintended consequences and the implications of genetic modifications.
- **Regulation:** Navigating the regulatory landscape for genetic modifications and ensuring compliance with ethical standards and safety protocols.
- These advancements in genomics and genetics hold great promise for improving animal health, productivity and welfare. They also offer opportunities to address global challenges such as food security, disease management and environmental sustainability.

2. Precision Livestock Farming (PLF) is a field focused on utilizing advanced technologies to monitor, manage and optimize livestock production on an individual animal basis [7]. The goal is to improve efficiency, welfare and sustainability in animal agriculture. Here's an overview of the key components and emerging trends in PLF:

2.1. Monitoring technologies

- **Wearable sensors:** Devices like GPS collars, accelerometers, and heart rate monitors provide real-time data on animal movement, behavior and health. These sensors can track physical activity, grazing patterns and even detect early signs of illness.
- **Automated feed systems:** Technologies that deliver precise amounts of feed based on individual animal needs and monitor feed consumption. This helps in optimizing nutrition and reducing waste.
- **Environmental sensors:** Instruments that measure environmental conditions such as temperature, humidity and air quality in animal housing. These sensors help ensure optimal living conditions and identify potential issues [8].

2.2. Data integration and analytics

- **Big data and machine learning:** Advanced analytics tools process vast amounts of data from sensors and other sources to provide actionable insights. Machine learning algorithms can predict health issues, optimize feeding schedules and improve breeding decisions.
- **Decision support systems:** Software platforms that integrate data from various sources to assist farmers in making informed decisions. These systems can recommend actions based on real-time data and historical trends.

2.3. Health and welfare monitoring

- **Health diagnostics:** Automated systems that use data from sensors and wearable devices to monitor vital signs and detect anomalies, such as changes in heart rate, activity levels, or temperature, which may indicate illness.
- **Behavioral analysis:** Using video cameras and image recognition software to analyze animal behavior, detect signs of distress and ensure that animals are engaging in normal behaviors [9].

2.4. Precision breeding

- **Genetic profiling:** Combining genomic data with real-time performance data to make more informed breeding decisions. This helps in selecting animals with desirable traits and improving genetic diversity.
- **Performance tracking:** Monitoring growth rates, reproductive performance and other key metrics to fine-tune breeding programs and enhance productivity.

2.5. Resource efficiency

- **Optimized feed use:** Precision feeding systems that adjust rations based on individual animal needs, improving feed efficiency and reducing costs. This also helps in minimizing the environmental impact of feed production.
- **Water management:** Technologies to monitor and control water consumption, ensuring that each animal has access to adequate hydration while reducing waste.

2.6. Sustainability and environmental impact

- **Manure management:** Automated systems for managing and processing manure, which can reduce environmental pollution and improve nutrient recycling.
- **Emission monitoring:** Sensors and analytics to track greenhouse gas emissions from livestock operations, helping to develop strategies for reduction and compliance with environmental regulations.

2.7. Automation and robotics

- **Milking robots:** Automated milking systems that can milk cows at any time, monitor milk quality, and manage udder health. This technology reduces labor requirements and improves efficiency.
- **Feeding robots:** Robots that deliver feed, clean feeding areas, and ensure even distribution, reducing manual labor and optimizing feed management [10].

2.8. Consumer and market insights

- **Traceability:** Technologies that track and record the entire lifecycle of animal products, from farm to table. This enhances transparency, food safety, and consumer trust.

- **Demand forecasting:** Analyzing market trends and consumer preferences to align production with demand, reducing waste and improving profitability.

2.9. Ethical and practical considerations

- **Animal welfare:** Ensuring that technologies used in PLF do not compromise animal welfare. Monitoring systems should be designed to improve conditions and provide humane treatment.
- **Data security and privacy:** Protecting the data collected from livestock and farm operations to prevent misuse and ensure compliance with privacy regulations.

2.10. Adoption and implementation

- **Cost and accessibility:** Addressing the costs of implementing advanced PLF technologies and making them accessible to farmers of all sizes, including small and medium-scale operations.
- **Training and support:** Providing education and support to farmers to effectively use PLF technologies and interpret the data generated.

3. Animal welfare and ethics are critical aspects of animal science that focus on ensuring the humane treatment of animals, both in agricultural settings and as companions. This field encompasses a range of concerns, from the daily care of animals to broader ethical questions about their role in society [11]. Here's an in-depth look at key issues and advancements in animal welfare and ethics:

3.1. Principles of animal welfare

- **Five freedoms:** A widely recognized framework for animal welfare that includes:
- **Freedom from hunger and thirst:** Ensuring access to fresh water and a nutritious diet.
- **Freedom from discomfort:** Providing a suitable environment, including shelter and a comfortable resting area.
- **Freedom from pain, injury, and disease:** Implementing preventive measures and providing veterinary care.
- **Freedom to express normal behaviour:** Allowing space and facilities for natural behaviours and social interactions.
- **Freedom from fear and distress:** Ensuring that animals are not subjected to unnecessary stress or fear [12].

3.2. Housing and environmental enrichment

- **Enriched environments:** Designing housing systems that mimic natural habitats and provide opportunities for species-specific behaviours. For example, providing perches for birds or rooting materials for pigs.

- **Space requirements:** Ensuring that animals have adequate space to move, socialize, and express natural behaviours, reducing overcrowding and stress.

3.3. Health and veterinary care

- **Preventive health measures:** Implementing vaccination programs, regular health checks, and biosecurity practices to prevent disease and promote well-being.
- **Pain management:** Providing adequate pain relief for procedures and conditions that cause suffering, including the use of anaesthetics and analgesics.

3.4. Ethical considerations

- **Animal rights vs. Animal welfare:** Balancing the ethical considerations of animal rights (the intrinsic value of animals) with the practical aspects of animal welfare (ensuring good living conditions).
- **Sentience:** Recognizing animals as sentient beings capable of experiencing pain, pleasure, and emotional states and adjusting practices accordingly.

3.5. Welfare in production systems

- **Humane slaughter:** Ensuring that animals are slaughtered in a manner that minimizes suffering, such as using stunning methods to render animals unconscious before slaughter.
- **Sustainable practices:** Implementing farming practices that reduce environmental impact and promote the well-being of animals, such as rotational grazing and integrated pest management.

3.6. Research and experimentation

- **3Rs principle:** Adopting the principles of Replacement (using alternatives to animal testing), Reduction (using fewer animals), and Refinement (improving methods to minimize suffering) in scientific research.
- **Ethical review boards:** Ensuring that animal research is conducted ethically and with approval from institutional review boards that evaluate the necessity and humane treatment of animals.

3.7. Companion animal welfare

- **Preventive care:** Promoting regular veterinary visits, vaccinations, and preventive treatments to ensure the health and well-being of pets.
- **Behavioural issues**: Addressing behavioural problems through training and enrichment to prevent neglect and improve the quality of life for companion animals [13].

3.8. Public awareness and education

- **Promoting welfare standards:** Educating the public and industry stakeholders about animal welfare standards and the importance of humane treatment.

- **Consumer choices:** Encouraging informed choices by consumers regarding animal products, such as supporting products from farms that adhere to high welfare standards.

3.9. Legislative and policy frameworks

- **Animal welfare legislation:** Advocating for and adhering to laws and regulations designed to protect animal welfare, including standards for housing, handling, and care.
- **International standards:** Contributing to and complying with international guidelines and agreements on animal welfare, such as those set by the World Organisation for Animal Health (OIE).

3.10. Challenges and future directions

- **Ethical dilemmas:** Navigating complex ethical dilemmas, such as the balance between animal welfare and agricultural productivity or scientific research.
- **Global variability:** Addressing differences in animal welfare standards and practices across regions and working towards global improvements.
- Advancements in Technology: Leveraging new technologies, such as precision farming and behavioural monitoring, to enhance animal welfare while ensuring ethical practices.

4. Sustainable practices in animal science focus on optimizing animal production systems to minimize environmental impact, ensure resource efficiency, and promote long-term ecological balance. Here's an in-depth look at key aspects and strategies for implementing sustainable practices in animal agriculture:

4.1. Sustainable feed and nutrition

- **Alternative feed sources:** Utilizing alternative feed ingredients like insect meal, algae, and by-products from agriculture or food industries to reduce reliance on traditional feed sources and lower the environmental footprint.
- **Feed efficiency:** Improving feed conversion ratios to ensure animals get the most out of their feed, reducing waste, and minimizing feed production impacts.
- **Precision nutrition:** Tailoring diets to the specific needs of individual animals or production systems to enhance nutrient use efficiency and reduce excess waste [14].

4.2. Resource management

- **Water use:** Implementing water-efficient practices, such as using low-flow watering systems, recycling water, and monitoring water quality to reduce consumption and waste.

- **Energy efficiency:** Adopting renewable energy sources (e.g., solar, wind), optimizing energy use in facilities, and improving insulation and heating systems to reduce energy consumption.

4.3. Waste management

- **Manure management:** Utilizing systems for composting, anaerobic digestion, or other methods to process manure into valuable by-products like compost or biogas, reducing environmental pollution.
- **Nutrient recycling:** Implementing practices to recycle nutrients from waste products back into the soil as fertilizers, which can reduce the need for synthetic fertilizers and improve soil health.

4.4. Land use and soil management

- **Sustainable grazing:** Employing rotational grazing and agroforestry practices to maintain soil health, prevent overgrazing, and enhance biodiversity.
- **Soil conservation:** Using techniques like cover cropping, reduced tillage, and buffer strips to prevent soil erosion, improve soil fertility, and increase carbon sequestration.

4.5. Emission reduction

- **Greenhouse gas reduction:** Implementing strategies to lower methane and nitrous oxide emissions from livestock, such as improving feed efficiency, using feed additives that reduce methane production and managing manure effectively.
- **Carbon footprint monitoring:** Tracking and reducing the carbon footprint of animal production through improved practices, technologies, and energy use.

4.6. Animal welfare and ethics

- **Humane treatment:** Ensuring that animal welfare standards upheld including providing adequate housing, veterinary care, and enrichment to promote the well-being of animals.
- **Ethical practices:** Adopting practices that respect animal rights and ensure that production systems are humane and environmentally responsible.

4.7. Biodiversity conservation

- **Habitat preservation:** Protecting natural habitats and biodiversity by avoiding deforestation and maintaining ecological balance within production systems.
- **Agroecological practices:** Integrating diverse crops and livestock species into farming systems to enhance resilience and support ecological diversity.

4.8. Sustainable aquaculture

- **Responsible feed use:** Using sustainably sourced fishmeal and other feed ingredients to minimize the impact on marine ecosystems.

- **Effluent management:** Implementing systems to manage and treat effluents from aquaculture operations to reduce pollution and impact on surrounding water bodies [15].

4.9. Technology and innovation

- **Precision agriculture:** Employing technologies like sensors, data analytics, and automation to optimize resource use, improve efficiency, and reduce waste.
- **Research and development:** Investing in research to develop new sustainable practices, improve animal genetics, and enhance production systems.

5. Reproductive technologies in animal science are advanced methods used to enhance reproduction, improve genetic quality, and manage breeding programs more effectively. These technologies are applied across various animal species, from livestock to companion animals, and play a crucial role in enhancing productivity, genetic diversity, and overall herd or flock management. Here's a detailed overview of key reproductive technologies:

5.1. Artificial Insemination (AI)

- **Overview:** AI involves the introduction of sperm into a female's reproductive tract without natural mating. It allows for the efficient use of superior male genetics and can be used in various species [16].
- **Benefits:** Increases the genetic diversity of a breeding program, reduces the need for maintaining a large number of breeding males, and enables the use of high-quality genetics across a wide geographical area.

5.2. Embryo Transfer (ET)

- **Overview:** ET involves collecting embryos from a donor female and transferring them to recipient females. This technology accelerates genetic improvement by allowing one female to produce multiple offspring.
- **Techniques:**
  - **Superovulation**: Stimulating a donor female to produce multiple eggs in one cycle.
  - **Embryo collection and transfer:** Harvesting embryos and transferring them to synchronized recipient females.
- **Benefits:** Enhances reproductive efficiency, spreads desirable genetic traits rapidly, and improves herd or flock genetics [17].

5.3. *InVitro* fertilization (IVF)

- **Overview: IVF** involves fertilizing eggs outside the body in a laboratory setting and then transferring the resulting embryos into a female's reproductive tract.
- **Techniques:**
  - **Oocyte collection**: Harvesting eggs from the female's ovaries.

- ***InVitro* fertilization**: Fertilizing the eggs with sperm in a controlled environment.
- **Embryo transfer:** Transferring embryos into a recipient female or incubating them further before transfer.

➢ **Benefits:** Allows for the use of eggs and sperm from individuals that may not be able to mate naturally, and facilitates genetic improvements [18].

5.4. Gamete and embryo cryopreservation

➢ **Overview:** Cryopreservation involves freezing and storing gametes (sperm and eggs) or embryos for future use. This technology preserves genetic material for breeding programs and research.

➢ **Benefits:** Enables the long-term storage of valuable genetics, facilitates international exchange of genetic material, and helps manage breeding programs.

5.5. Genetic sexing

➢ **Overview:** Genetic sexing techniques determine the sex of embryos or sperm, allowing for the production of offspring of a desired sex.

➢ **Techniques:**

- **Sexed semen:** Sorting sperm cells based on sex chromosomes before artificial insemination.
- **Embryo sexing:** Identifying the sex of embryos before transfer.

➢ **Benefits:** Useful in species where sex-specific traits are valuable, such as dairy cattle (female calves for milk production) or beef cattle (male calves for meat production).

5.6. Cloning

➢ **Overview:** Cloning involves creating a genetically identical copy of an animal through techniques such as somatic cell nuclear transfer (SCNT).

➢ **Benefits:** Allows for the replication of individuals with desirable traits, preserves endangered species, and aids in research.

5.7. Hormonal manipulation

➢ **Overview:** Hormonal treatments are used to synchronize estrus (heat) cycles, induce ovulation, and manage reproductive processes.

➢ **Techniques:**

- **Estrus synchronization:** Administering hormones to synchronize estrus cycles among females.
- **Induction of ovulation:** Using hormones to stimulate ovulation in females.

➢ **Benefits:** Improves the timing of breeding, enhances reproductive efficiency, and facilitates artificial insemination.

5.8. Reproductive management software

- **Overview:** Software tools help manage and monitor reproductive programs, track breeding records, and analyze data to optimize reproductive efficiency.
- **Benefits:** Provides valuable insights into reproductive performance, assists in decision-making, and improves overall management of breeding programs.

5.9. Assisted Reproductive Technologies (ARTs)

- **Overview:** ARTs encompass various techniques designed to assist with reproduction, including AI, IVF, ET, and gamete cryopreservation.
- **Benefits:** Enhances reproductive success, facilitates genetic improvements, and supports conservation efforts [19].

5.10. Advances and innovations

- **Genomic tools:** Using genetic and genomic tools to identify optimal breeding pairs, enhance genetic selection, and improve reproductive outcomes.
- **Precision breeding**: Integrating data from reproductive technologies, genetic testing, and performance metrics to optimize breeding strategies and outcomes.

6. Innovation in animal products encompasses advancements in how animal-derived products are developed, processed, and marketed. These innovations aim to improve quality, enhance sustainability, and meet evolving consumer demands. Here's an overview of some key areas of innovation in animal products:

6.1. Enhanced production techniques

- **Precision livestock farming:** Utilizing technologies like sensors, GPS, and data analytics to monitor animal health, optimize feeding, and improve overall production efficiency.
- **Automated systems:** Implementing automated milking systems, feeding robots, and waste management technologies to enhance productivity and reduce labour.

6.2. Genetic improvement

- **Selective breeding:** Using advanced genetic tools to select and breed animals with desirable traits, such as improved growth rates, disease resistance and better feed conversion.
- **Gene editing:** Applying techniques like CRISPR-Cas9 to modify animal genomes to enhance traits, such as disease resistance or improved meat quality.

6.3. Sustainable practices

- **Alternative feeds:** Incorporating sustainable feed sources, such as insect meal, algae, or by-products from other industries, to reduce reliance on traditional feed resources and lower environmental impact.

- **Waste reduction:** Developing technologies to convert animal waste into valuable by-products like compost or biogas, reducing environmental pollution and enhancing resource efficiency.

6.4. Novel animal products

- **Lab-grown meat:** Producing meat from cultured cells in a laboratory setting, offering a sustainable alternative to traditional meat production with reduced environmental impact and ethical concerns.
- **Plant-based alternatives:** Creating plant-based products that mimic the taste, texture, and nutritional profile of animal products, catering to the growing demand for vegetarian and vegan options.

6.5. Quality and safety enhancements

- **Food safety technologies:** Implementing advanced food safety measures, such as improved testing methods for pathogens and contaminants, to ensure the safety and quality of animal products [20].
- **Traceability:** Using blockchain and other technologies to enhance transparency and traceability in the supply chain, ensuring the authenticity and safety of animal products.

6.6. Nutritional enhancements

- **Biofortification:** Enhancing the nutritional content of animal products through dietary modifications or genetic improvements, such as increasing the omega-3 fatty acids in meat or dairy products.
- **Functional foods:** Developing animal products with added health benefits, such as probiotics in dairy products or fortified meats with vitamins and minerals.

6.7. Processing innovations

- **Advanced processing techniques:** Utilizing new processing methods, such as high-pressure processing or advanced refrigeration, to extend shelf life, enhance safety, and improve the quality of animal products.
- **Sustainable packaging:** Exploring eco-friendly packaging solutions, such as biodegradable materials or reduced plastic use, to minimize environmental impact.

6.8. Consumer experience

- **Customization:** Offering personalized animal products tailored to individual dietary needs or preferences, such as customized meat cuts or dairy products with specific flavor profiles.
- **Convenience products:** Developing ready-to-eat or easy-to-prepare animal products that cater to busy lifestyles and changing consumer preferences.

6.9. Animal welfare improvements

- **Welfare-friendly practices:** Innovating in animal husbandry to improve welfare conditions, such as enhancing living environments, providing enrichment, and reducing stress through better management practices.
- **Ethical sourcing:** Emphasizing humane and ethical sourcing practices, including certifications for animal welfare and sustainable farming methods.

6.10. Research and development

- **Collaborative research:** Partnering with research institutions, universities, and industry stakeholders to drive innovation in animal product development and address emerging challenges.
- **Investment in R&D:** Supporting research and development initiatives to explore new technologies, improve existing products, and develop novel solutions for animal agriculture.

6.11. Market trends and consumer preferences

- **Health-conscious choices:** Responding to consumer demand for healthier, lower fat, and higher-quality animal products by developing new formulations and product lines.
- **Ethical and sustainable products:** Meeting the growing consumer interest in ethically produced and environmentally friendly animal products through innovation and transparency.

6.12. International and regulatory considerations

- **Global standards:** Navigating international regulations and standards for animal products to ensure compliance and facilitate global trade.
- **Regulatory innovation:** Adapting to evolving regulations and standards related to new technologies and product innovations in animal agriculture.

Conclusion:

These areas represent a snapshot of the dynamic and multidisciplinary nature of animal science research and development. Each of these frontiers has the potential to make significant impacts on animal health, productivity, and welfare, as well as on broader environmental and societal issues.

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THE IMMORTAL JELLYFISH: UNDERSTANDING THE UNIQUE LIFE CYCLE AND BIOLOGICAL IMMORTALITY

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Abstract:

Turritopsis nutricula, often known as the immortal jellyfish, displays a remarkable biological capacity to revert to its juvenile polyp stage after achieving sexual maturity, essentially allowing it to escape death and perhaps achieve biological immortality. This extraordinary mechanism, known as transdifferentiation, permits differentiated adult cells to convert into different cell types, enabling regeneration. Following reproduction, Turritopsis nutricula undergoes considerable morphological changes, reabsorbing its exterior components to create a cyst-like structure that eventually grows into a colony of polyps. These polyps can grow into genetically identical medusae, thereby continuing the life cycle. This cyclical rejuvenation, which might theoretically occur endlessly, is unique among multicellular animals and has gained scientific interest for its implications on aging and regeneration. The jellyfish's capacity to adapt to environmental challenges by returning to an earlier life stage may reveal insights on cellular regeneration and lifespan in other animals, including humans.

Introduction:

The hydrozoan specie *Turritopsis nutricula* is unique individual which when reaches sexual maturity, it may return to a sexually immature polyp stage, making it biologically immortal. A non-stem cell can change into a new type of cell with different functionality through a process known as transdifferentiation. Understanding the connection between transdifferentiation and stem cells is essential to comprehending *Turritopsis nutricula* longevity, even if the exact role of stem cells in this phenomenon is yet unknown. Although transdifferentiation is uncommon, it is a part of the *Turritopsis* life cycle and enables it to regenerate and start living again as a juvenile polyp. This special ability may provide light on human biological immortality. Transdifferentiation is very rare, and when it does occur, it most usually occurs in portions like in specific organs or tissue of the organisms, such in the eye of the salamander, Hydra, alpha and beta cells of human pancreas, lobsters, etc. At the end of the life cycle, the immortal jellyfish (*Turritopsis sp.)* is a juvenile polyp, which is ready to start its life again (Martell *et al.*, 2016; Bavestrello *et al.*, 1992; Piraino *et al.*, 1996).

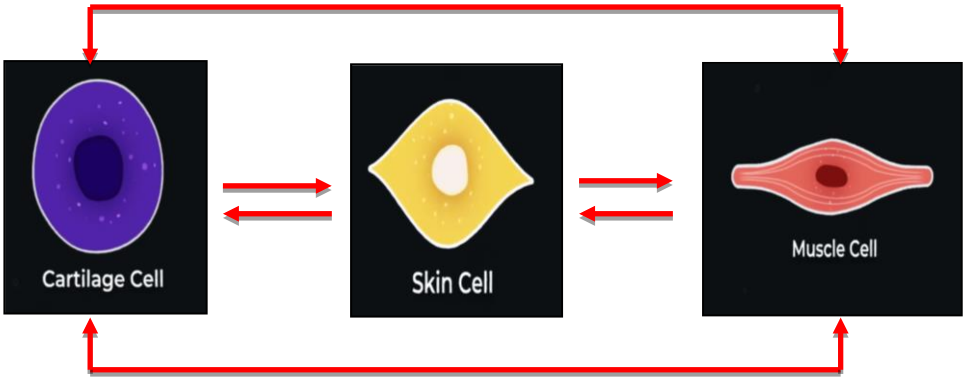

Fig. 1: Cellular Transdifferentiation

Description:

Turritopsis nutricula has a diameter of about 5 mm. It has bell shaped and height and breadth is nearly equal. The walls are all the same thinness, with thicker middle portion. The cross section of the large, brilliant red stomach is cruciform. Adult individuals exhibit 80–90 tentacles around the edge, compared to just 8 on young ones. Most jellyfish die after reproducing, *Turritopsis nutricula* may regenerate itself and returns to a sexually premature stage after reaching adulthood. Scientists and marine biologists are currently focusing their research on this jellyfish to determine how exactly this jellyfish reverse the aging process. It is assumed that transdifferentiation - the process by which cells change from one kind to another - is how it accomplishes the feat.

Fig. 2: *Turritopsis nutricula*

These jellyfish represent the first known example of a metazoan, or multicellular organism, exhibiting the capacity to return to a colony stage following sexual maturity. But *Turritopsis nutricula* change, going from a sexually immature state to a mature state after each reproduction cycle, then back to a sexually immature state, and so on. Most animals eventually die after they have reproduced sexually. Transdifferentiation is the process by which one kind of cell changes into another entirely. Similar to the widely reported stem cells, these cells have the ability to completely alter their composition through transdifferentiation (Piraino *et al.*, 1996). The jellyfish reabsorbs all its exterior components after sexual reproduction and becomes a cyst, which resembles a blob resembling an ameba. After that, the cyst clings to the earth and develops into a colony of stalk-shaped polyps. These polyps start a new cycle in which they develop into fully grown, genetically identical jellyfish (Kubota *et al.*, 2011).

Classification

Kingdom – Animalia

Phylum – Cnidaria

Class – Hydrozoa

Order – Hydroida

Family – Clavidae

Genus – Turritopsis

Species – *Turritopsis lata*, *T. nutricula*, *T. fascicularis*, *T. dohrnii*, *T. minor*, *T. rubra*, *T. pacifica.*

Life cycle

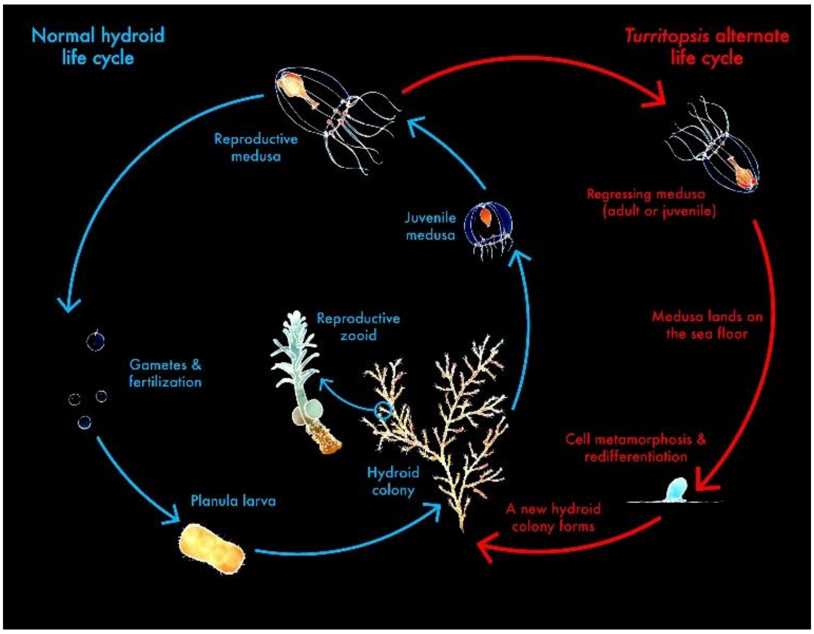

Fig. 3: Life Cycle of *Turritopsis sps*.

The fertilized eggs grow in the stomach and in the screen provided by the cave in the jellyfish planula. The eggs are subsequently placed on the bottom in polyp colonies. The jellyfish spawns after two days. The jellyfish gets sexually mature after a few weeks (the precise length depends on the water temperature; at 22°C it is 18-22 days while at 20°C it is 25-30 days). It is the sole known creature that can return to its juvenile polyp stage. Theoretically, the process can repeat endlessly, rendering it potentially eternal. these species are found in tropical region and is thought to be spreading around the planet when ships' bilge water is dumped at ports. Though solitary, they are carnivorous organisms that grow asexually from a polyp stage.

The medusa changes into a stolon (cyst) and the polyps become a hydroid colony in the *Turritopsis nutricula* life cycle. Before the polyp spawns, the primary part and tentacles of the umbrella turn inside out. Two days prior to the polyps differentiating, stolons (cysts) develop (Yamada *et al.*, 1971). The *Turritopsis nutricula* become physiologically immortal as a result of the jellyfish's capacity to reverse the life cycle, which is presumably unique in the animal realm. It is able to accomplish this because it has the ability to change a cell's differentiated status and turn it into a different type of cell. This process is known as transdifferentiation, and it is often only observed when organ parts regenerate. Nonetheless, it seems to happen regularly during the life cycle of *Turritopsis nutricula*. The medusa undergoes transdifferentiation to become the stolons and polyps of a hydroid colony. Prior to the mesoglea and tentacles being resorbed, the umbrella everts. Spawning happens soon after the everted medusa connects to the substrate by the end that was at the other end of the umbrella. After that, the cnidarian secretes stolons (cysts) and a perisarc. Polyps differentiate two days after the cysts are initially seen. These polyps begin blossoming off new medusae and usually feeds on zooplankton.

Fig. 4: Polyp Stage

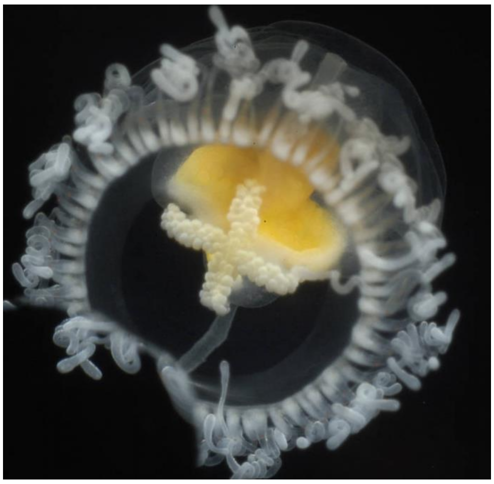

Fig 5. Medusa Stage

Working of cellular Transdifferentian in *Turritopsis nutricula*

In complete life cycle of *Turritopsis nutricula*, cyst stage has most important role to play. Cyst stage is totally focused on spending good amount of energy in carrying out DNA work. DNA work includes, repairing of Telomeres (chromosome tips/ Strands of DNA found at tip of each chromosome). Telomeres protects DNA from getting damaged, which generally gets damaged during cell replications. In animal kingdom every time the cells replicate some amount of DNA is lost from the telomeres, which leads to telomere shortening which ultimately lead to cell death, but this is not in case of *Turritopsis nutricula*. Cyst of *Turritopsis nutricula* contains very high number of genes that promotes telomerase enzyme production (Which repairs telomeres from getting damaged). Whole animal kingdom, (except Genus *Turritopsis*) contains very few numbers of these telomerase enzyme. Thus, with large numbers of telomerase enzyme *Turritopsis nutricula* can protects its chromosomes from getting damaged by simply elongating the telomers every time cell replicates and ultimately avoiding Senescence process. Thus, during cyst stage the only role cyst play is to go on producing telomerase enzymes and work on DNA repair and maintenance, until it ready to become polyp once more. In *Turtitopsis* species gastrovascular cavity fragments is functional dominant in the regulation of cellular responses to external, or internal stress, whereas the nerve ring fragment may contribute to developmental initialization processes and serve as a central nervous-like system with possibility of stem cell existence acting as a placenta-like tissue (Liu *et al.,* 2024).

Production of enzyme / proteins

The key steps of transcription are:

1. Initiation: RNA polymerase, the enzyme that catalyzes transcription, binds to a promoter sequence on the DNA to mark the start site of transcription.
2. Elongation: RNA polymerase unwinds the DNA double helix and uses one strand as a template to synthesize a complementary RNA molecule. Ribonucleotides are added to the growing RNA strand in a 5' to 3' direction.
3. Termination: RNA polymerase encounters a terminator sequence on the DNA, which signals it to stop transcription and release the newly synthesized mRNA (used as template for making protein/enzyme).

- After which mRNA enters cytoplasm, where it gets attached by 80S ribosome.
- Ribosome reads three bases at a time and simultaneously instruct tRNA to bring required amino acid. Amino acid chain is formed at end when whole mRNA sequence is read.
- Then this Amino acid is chain is folded into specific shape and is assigned with specific role which is termed as enzyme.

Conclusion:

The application of a study of the *Turritopsis nutricula* could be boundless, as stem-cell research appears at the forefront of many medical studies on organ reproduction, cancer treatments, and brain injury treatments to name a few. By using the cells of the jellyfish, which transdifferentiate, scientists can continue to research solutions for these problems without mucking about in the moral dilemmas that come with researching embryonic stem cells. The jellyfish’s cells are also similar in make to cancer cells, which can affect the order and process of genetic systems. By studying these cells, scientists may be able to gain insight in the never-ending search for a cure for cancer. It is believed that they spread when the jellyfish stow away in the ballast tanks of large ships and are carried from place to place.

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EXPLORING THE BOUBA KIKI PARADIGM AMONG THE POPULATION OF A METROPOLITAN CITY

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Abstract:

The Bouba-Kiki (BK) paradigm is the cross-modal congruency connecting certain speech sounds with certain geometrical patterns. This comprises a singular deviation to the prevalent chance of the relatedness between word denotation and word sounds. We attempted an online experiment across a diverse population in the city of Mumbai regarding Bouba-kiki effect. The study was conducted via dissemination of Google forms through social media to assess the prevalence of this phenomenon in different subgroups of non-synaesthetic and non-autistic participants (n=297). We investigated the influence of vowels and consonants in a word-shape association task. A significant influence of vowels was observed in word-shape association tasks. Also, a relationship was found among gender and the preference of vowels. Our results indicate~~d~~ a statistically notable difference between the mean total congruent scores in preference of vowels (2.37 ± 0.82 vs 2.06 ± 1.006, p-value = 0.04) between male and female participants.

Keywords: Language, Word, Shape, Taste, Word –Shape Association, Gender

Introduction:

The “bouba-kiki effect” is a non-arbitrary tallying of the word kiki with a craggy star shape and bouba with an ovoid orbicular shape. This effect was first noticed by Wolfgang Köhler in 1929, with two analogous terms, baluba and takete, and followed with maluma and takete. He observed that most people chose “baluma” for the ovoid shape and “takete” for the serrated one. This aspect was further explored by Ramachandran & Hubbard in 2001 with the words bouba and kiki. Their experiments brought to light an intense and consensual link between idle speech sounds and geometrical configurations, an actuate now cited as the bouba-kiki effect. This portraying is not only limited to the non-words bouba and kiki, but also stretches to various other phonemes. This function is very likely the most prevalent in the field of sound symbolism. Multitude of studies show the same model of response in a 9 to 1 manner. Similar effects have been displayed by inhabitants from countries speaking nonidentical languages, in response to varied kinds of phonetic and visual materials (De Carolis *et al.*, 2018). Several studies report that the native languages are emblematic sounds that people relate to concepts (Ćwiek *et al.*, 2021; Ramachandran & Hubbard, 2001). In due course these concomitant sounds grew more varying and structurally elaborate as they advanced into more complex speech. This brought up a major question: Were the initial sounds made by individuals arbitrary and random, or were they invariably related to certain metaphors and concepts? This phenomenon, the Bouba–Kiki effect could possibly help us find out more about the evolution of language, in some ways.

Cuskley *et al.,* (2013) investigated the phonological and orthographic influences in the bouba-kiki mapping task. Most of the detailed evaluations reveal major influence of acoustic or articulatory features of sound to be the intrinsic mechanism, however their study acknowledges that the conformity based on letter curvature and shapes (mapping ovoid shapes to non words with curviform letters) is the dominant mechanism observed among literate individuals. Likewise, studies have shown that consonants play a more governing role than vowels in an opted association (Fort *et al.,* 2015). (Sidhu and Pexman, 2017) demonstrated the effect of sound symbolic priming where the participants were more likely to make an accurate grading following a congruent nonword prime than an incongruent nonword prime with regards to the shape. They gave the evidence that the phonemes associated with orb-shaped or spikiness are typically present in the words that refer to round objects or spiky objects respectively. (Wilkinson *et al.,* 1998) explored the effect of features of visual stimuli in shaping people's response by manipulating three factors of radial frequency patterns, namely frequency, amplitude, and spikiness. They concluded that participant responses were more inclined towards "Kiki" to enhanced modes of the visual stimuli. Chen *et al.* (2016) replicated the same experiment to analyze the confidence of participants while making non cognitive judgments regarding cross modal correspondence. They achieved that the confidence ratings of the participants were higher when their judgments were consistent with major responses of the group, especially for patterns predominantly associated with "Kiki". Experiments conducted by Gallace *et al.,* (2011) suggest that the bouba kiki type associations also continue towards word-food associations. It is interesting to note that studies by the same group found that individuals routinely conformed about which foods seemed more kiki and which more bouba. For example, carbonated beverages, dark chocolate, sharp cheese, and Bitburger beer, were inferred to be more kiki; milk chocolate and Brie cheese more bouba.

Many studies have been conducted to analyze the crossmodal association among the general population (Bremner *et al.,* 2013). In this paper, we recreated the experiment performed by (De Carolis *et al.,* 2018) on the population of Mumbai, predominantly an urban area with some modifications. Mumbai city is a large and densely populated metropolis. We were curious to observe the phenomenon of Bouba-Kiki among the inhabitants of the city. Due to the presence of people belonging to varied linguistic sects, speaking a number of languages with varied dialects and belonging to different ethnic groups cohabiting the city, deviations from what has been observed in the previous studies may be possible. Hence, the experiment was carried out to examine the extent of the existence of the Bouba-kiki phenomenon in different age groups of non synaesthetic and non autistic urban participants. We also investigated the influence of vowels and consonants in a word-shape association task among this multi-cultural group. We aimed to further explore this aspect of sound–shape matching tasks, in particular among the genders, and delineate if any, possible difference in approach towards the phenomenon of Bouba-Kiki.

Method:

Study design:

An online survey via Google forms was carried out for this study and the questionnaire was circulated in the general population via social media in the time period of November 2021 to March 2022. The Google forms were designed to inform the participants about the intended study. All participants were pre-informed regarding the objective of the study and informed consent was taken. This was included in the google form and participants' consent were obtained before they proceeded to attempt any of the questions linked to the study. No personal information was collected and the entire process was carried out keeping anonymity as priority. The participation in this survey was entirely voluntary and without any incentives.

There were 41 questions in the google form in all, including the personal data collection questions, for maintaining a record and eliminating the synaesthetes and autistic respondents. In the questionnaire, a total of 29 questions in which 12 questions involving 2x2 association (2 words and 2 figures) and 7 questions in 1x2 (2 figures - 1 words), 2 questions in 1x2 (2 words - 1 figure) were framed where words with different patterns of vowels and consonants were used, some of them were from the supplementary data by (De Carolis *et al.*, 2018). The remaining 8 questions were drafted out to investigate the word-taste association (5 questions) and shape - taste association (3 questions) The questions were presented in a mixed format. The stimuli provided were in the form of visual stimuli including pictures and written words. No auditory stimuli were provided. The questions were framed in a manner that enables us to validate the presence of Bouba-kiki effect in the population under study and analyze the effects of vowels and consonants. Among the questions asked via google forms, 15 questions were framed to check the presence of the bouba kiki effect in a word-shape association task. The questions were presented as a 2x2 association task and 1x2 association task. In the questions, two figures, one with a spiky frame and one with a round frame were presented and participants were asked to choose a word for each of them from two words given. These words had congruent combinations of vowels and consonants. Similarly, 8 questions were formulated for taste - word/shape association task, where participants were asked to choose between two shapes for the described taste or to choose a word for the described taste. Based on the given options, each congruent answer was assigned a score of 1 and incongruent score was assigned a score of 0. The total score of each individual was calculated and a paired Student's t-test was carried out between congruent and incongruent scores. The congruency of the word-shape association and taste - word/shape association tasks were interpreted from the available literature as shown in Table 1.

Table 1: Categories for evaluation

Parameters	Type of Association	
Frames X Consonants	Congruent : Spiky frames and voiceless plosives Round frames and sonorants	Incongruent : Round frames and voiceless plosives Spiky frames and sonorants
Frames X Vowels	Congruent Round frames and presence of lip rounding in o and u Spiky frames and absence of lip rounding in i and e	Incongruent spiky frames and presence of lip rounding in o and u Round frames and absence of lip rounding in i and e
Tastes X Vowels	Congruent: Citrusy and crisp food and front vowels (e.i) Sweet/smooth textured foods and back vowels(o.a)	Incongruent: Sweet/smooth textured food and front vowels (e.i) Citrusy and crisp foods and back vowels(o.a)
Frames X Tastes	Congruent: Spiky frames and Citrusy and crisp food Rounded frames and Sweet/smooth textured foods	Incongruent: Rounded frames and Citrusy and crisp food Spiky frames and Sweet/smooth textured foods

Statistical analysis:

Categorical data was represented as frequency and percentage. Statistical analysis was performed using R studio 4.2, Statistical Package for Social Sciences (SPSS) and Graphpad Prism v8.0.2. A Student's t-test was carried out between mean congruent score and mean incongruent score at a confidence level of 95%.

Results:

The respondents majorly belonged to urban areas. The responses were collected from 297 individuals that included 56 males and 241 females. Within the collected responses, two individuals were found to be synesthetic. The sample consists of individuals from all age groups, between the ages of 5 to 64, with the majority being in the age group of 15 - 24 y.o (n=181). The education level ranged from pre primary to the doctorate, though one respondent was a pre-reader. Ninety of the respondents completed graduation whereas fifty-six had finished Masters level of education. The ratio of male to female was 1:4 among the respondents. The description of the sample statistics is mentioned in Table 2.

Table 2: Descriptive statistics of the population sample

Parameter	**Value (n = 297)**
Age group	
5-14	27
15-24	181
25-54	84
55-64	3
Education	
Pre reader (children just beginning to read)	1
Pre school	2
Primary School	8
Middle School	15
High School	28
Junior College	89
Graduate	90
Masters	56
PhD	6
Sex	
Male/Female	56/241
Individuals diagnosed with Synaesthesia	2

The proportion of individuals and their responses to categories of the questionnaire was enumerated as in Table 3. In associations involving two visual stimulus (round frame and spiky frame) presented along with two pseudo words with congruent combinations of vowels and consonants (e.g Bouba and Kiki for the first question), there was a higher proportion of congruent responses, 221 out of 297 (Bouba chosen for round frame and Kiki for spiky frame) and lesser number of incongruent responses, 76 out of 297.

A set of questions were framed to investigate the effect of only consonants where there were two visual stimuli, one round frame and one spiky, whereas the two pseudowords presented differed only by consonants and had the same vowel, mostly in a consonant-vowel-consonant-vowel (CVCV) form.

Table 3: Survey questions, categories and proportion of individuals providing congruent and incongruent responses to the given questionnaire

Sl no.	Category	Question	Congruent	n (%)	Incongruent	n (%)
1	Vowel and Consonant	Label the objects as one 'Bouba' and another 'Kiki' based on what you think or feel	Kiki, Bouba	221	Bouba, Kiki	76
2	Consonants	Label the objects as one 'Utap' And Other 'Umam'.	Umam, Utap	199	Utap, Umam	98
3		Choose the words for given objects - Boub, Uomul	Boub, Uomul	114	Uomul, Boub	183
4		Choose the words for given objects - Ippipe, Innim	Innim, Ippipe	151	Ippipe, Innim	146
5	Vowel and Consonant	Which shape you associate with the following words - Kita Mosla	Kita, Mosla	227	Mosla, Kita	70
6	Consonants	Label the objects as one 'Ugug' and 'Umum' based on what you think or feel	Ugug, Umum	146	Umum, Ugug	151
7	Vowel and Consonant	Assign the words to the figures based on their sound 'Titi' 'Lulu'	Titi, Lulu	231	Lulu, Titi	66
8	Vowels	Read the words aloud 'Titi' 'Tutu' and assign them to the given figures	Titi, Tutu	214	Tutu, Titi	83
9		Assign the words to the figures based on their sound 'Lili' 'Tutu'	Tutu, Lili	210	Lili, Tutu	87
10		Assign the words to the figures based on their sound 'Lili' 'Lulu'	Lulu, Lili	207	Lili, Lulu	90

11	Consonants	Choose the words for the given object 'Popo' 'Lolo'	Lolo	124	Popo	173
12		Choose the words for the given object 'Gouda' 'Moulu'	Moulu	150	Gouda	147
13		Choose the words for the given object 'Mille' 'Tipi'	Mille	183	Tipi	114
14		Choose a picture for the word 'Mener'	B	130	A	167
15		Choose the words for the given object 'Titi' 'Nini'	Titi	198	Nini	99
16	Shape, word and taste	Which shape would you associate with a sweet or fatty taste	B	254	A	43
17		Which word would you associate with sharp, acidic and citrusy flavours	Touki	220	Malue	77
18		Which word would you associate with a sweet taste	Lumou	205	Itape	92
19		Which shape would you associate with sharp, acidic, citrusy taste	A	256	B	41
20		Which word would you associate with a fatty taste	B (Noul)	128	A (Pouki)	169
21		Which word would you associate with crisp, fresh flavors and tastes	A (Touca)	210	B (Nilou)	87
22		Which word would you associate with sweet fatty taste	A (Mosla)	181	B (Kitaki)	116
23		Which shape would you associate with crisp and fresh flavors and tastes	A	232	B	65

In this study, congruency is defined by round frames with sonorants (m,n) and spiky words with voiceless plosives (p,t). For example, in question 2 (Table 3), there were 199 congruent responses out of 297 and 98 incongruent responses out of 297. However, for similar kinds of questions (Q.3, Q.4, Q.6, Q.11) as shown in Table 2, the proportion of congruent and incongruent responses were nearly equal (congruent responses = 151/297, incongruent responses = 146/297 for Q.4)

We repeated the same pattern of investigation to assess the influence of vowels, where there were two visual stimuli, one round frame and one spiky, whereas the two pseudowords presented differed only by vowels and had the same consonant, in a CVCV form. Here, the congruency is defined by round frames with vowels like 'o and u' and spiky words with vowels like 'i and e'. For example, in question 8 (Table 3), there were 214 congruent responses out of 297 and 83 incongruent responses out of 297.

We found that there was a notable statistical difference between the mean total congruent scores and the mean total incongruent scores in the word-shape association task (p value < 2.2e-16 at 95% confidence interval). Hence, it can be inferred that the bouba kiki effect is prevalent in the population. Also a Student's t-test was done to test the difference between the mean of the total congruent scores and mean of the total incongruent scores in the taste - word/shape association task. The results indicated that there was a statistically significant difference (t = 18.832,p-value < 2.2e-16) between the mean total congruent scores and the mean total incongruent scores of the taste - word/shape association.

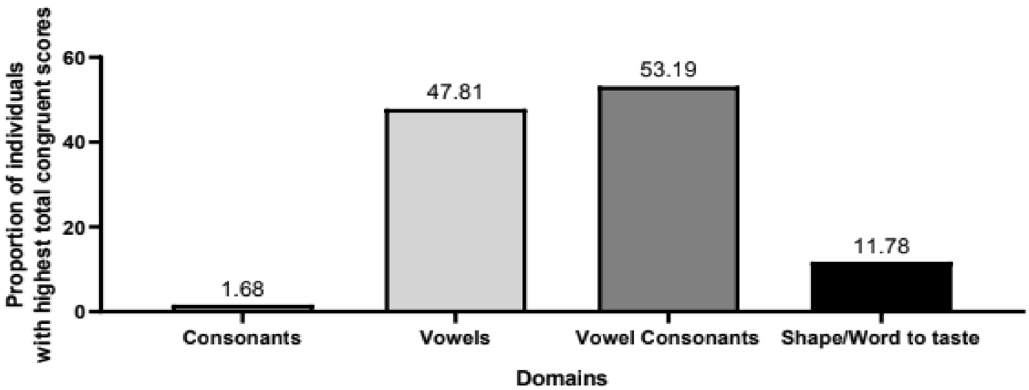

Figure 1: Total congruent scores and the mean total incongruent scores in the word-shape association task

As depicted by the graph in Figure 1, we found that individuals were less congruent in preference of consonants and shape/word to taste. Approximately, 50% of the study population were congruent in preference of only vowels and both vowels and consonants.

Subgroup analysis:

A subgroup analysis was conducted to understand the association between demographics and preference to the given domains.

Influence of vowels and consonants:

A paired t test was conducted between the mean congruent and non congruent scores of the questions investigating the effect of vowels (Q.8, Q.9, Q.10 - Table 3) The p value obtained is 0.001074 at 95% confidence interval, indicates that there is a statistical difference between the means of the congruent and incongruent scores. The congruent scores are significantly higher than the incongruent scores.

Also, a paired t test was carried out on the congruent and incongruent scores of the consonant category (Q.2, Q.3, Q.4, Q.6, Q.11, Q.12, Q.14, Q.15 - Table 3). The p value obtained at 95% confidence interval is 0.4257. This indicates that there is no statistically significant difference between the mean scores. Therefore, we can conclude that, in the case of consonants, the responses were not greatly influenced by the corresponding consonants of the psudowords presented.

A t test (unpaired) was carried out on the mean congruent score of consonants (158.1250) v/s mean congruent score of vowels (210.3333). The results yielded a significant p value of 0.002367 at 95% confidence interval. This indicates that there is a greater influence of vowels as compared to the influence of consonants.

Association between sex and preference of vowels:

We tried to assess the influence of vowels, where two visual stimuli were presented, one round frame and one spiky, whereas the two pseudowords presented differed only by vowels and had the same consonant, mostly in a CVCV form (Questions 17-21). Here, the congruency was defined by round frames with vowels like ‘o and u’ and spiky words with vowels like ‘i and e’. A question having pseudowords with incongruent consonant vowel combination was also tested in 2 X 2 association task to check for the influence of either one. The congruent and incongruent scores were evaluated and a comparison was done between the two sex as indicated in Table 4.a.

Table 4.a: Association between sex and preference of vowels among the population

Vowels	Male		Female		p-value
	Congruent	Non-congruent	Congruent	Non-congruent	
Titi - Tutu	45	11	169	72	0.124
Lili - Tutu	45	11	165	76	0.07
Lili- Lulu	43	13	164	77	0.20

Comparison of mean total congruent scores in preference of vowels between male and female participants

A chi-square test of independence was performed to examine the relation between sex and the preference of vowels on the data presented in table 4.a. The results as indicated in table 4.b, show that there was a statistically significant difference between the mean total congruent scores in preference of vowels between male and female participants, U = [5676], p = 0.046, as given in Figure 2.

Table 4b: Chi square test on overall mean score derived from association between sex and preference of vowels

Vowels	Mean (SD) Total congruent scores	p-value
Male	2.37 ± 0.82	0.04
Female	2.06 ± 1.006	

Association between sex and preference of consonants

Table 5a: Association between sex and preference of consonants among the population

Consonants	Male		Female		p-value
	Congruent	Non-congruent	Congruent	Non-congruent	
Umam, Utap	39	17	160	81	0.64
Boub, Uomul	19	37	95	146	0.44
Innim, Ippipe	32	24	119	122	0.44
Ugug, Umum	29	27	117	124	0.66
Lolo, Popo	27	29	97	144	0.76
Moulu, Gouda	32	24	118	123	0.27
Mille, Tipi	37	19	146	95	0.44
Titi, Nini	30	26	168	73	0.02*

Assessment of the influence of consonants (Questions 11-13, 15, 22, 24, 27, 34), where two visual stimuli, one round frame and one spiky, whereas the two pseudowords presented differed only by consonant and had the same vowels, for a 2 X 2 association task. The congruent and incongruent scores were evaluated and a comparison was done between the two sex as indicated in Table 5.a. A chi-square test of independence was performed to examine the relation between sex and the preference of consonants. The relation between these variables was statistically insignificant, except for question 15 (assigning Titi – Nini to object), where there was a significant association found, p = 0.02.

Comparison of mean total congruent scores in preference of consonants between male and female participants

An overall mean score from the data obtained from association between sex and preference of consonants was used for further analysis (Table 5.b).The results indicated that there was a statistically significant difference between the mean total congruent scores in preference of vowels between male and female participants, U = [6234], p = 0.36, as given in Figure 2.

Table 5b: Chi square test carried out on overall mean score derived from Table 5.a for assessment of preference for consonants between sex

Consonants	Mean (SD) Total congruent scores	p-value
Male	4.37 ± 1.25	0.36
Female	4.23 ± 1.47	

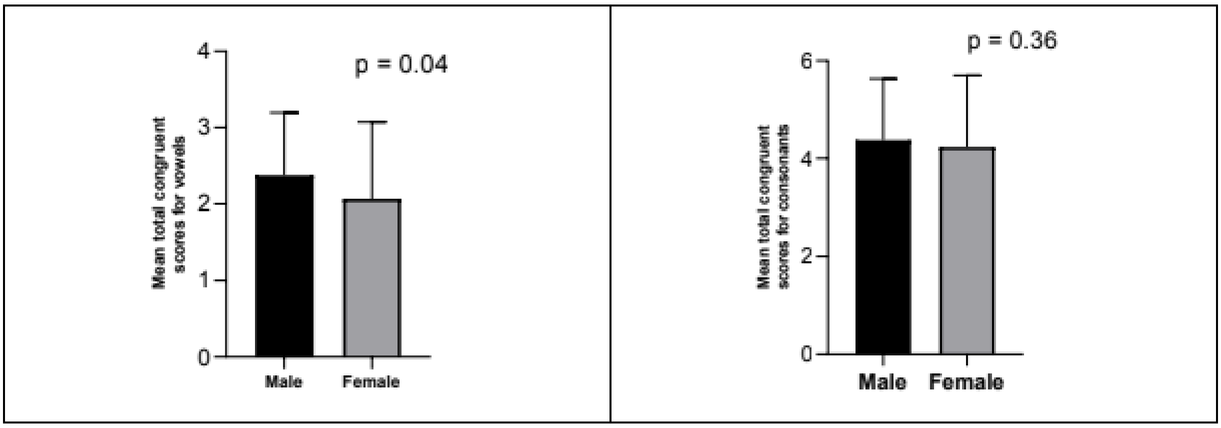

Figure 2: Mean total congruent scores for vowels association with gender from the study sample

Association between age groups and preference of vowels

An evaluation of the influence of vowels was done between age groups. Two age groups were considered, below 25 years old and above 25 years old. The congruent and incongruent scores were calculated for each of the two groups (Table 6.a) A chi-square test of independence was performed for each question to examine the relation between age groups and the preference of vowels. The relation between these variables was statistically insignificant.

Table 6a: Association between age groups and preference of vowels

Vowels	<25 years		≥25 years		p-value
	Congruent	Non-congruent	Congruent	Non-congruent	
Titi - Tutu	147	63	67	20	0.22
Lili - Tutu	150	60	60	27	0.67
Lili- Lulu	144	66	63	24	0.51

Comparison of mean total congruent scores in preference of vowels between age groups <25 years and ≥25 years

The mean scores from the association between age groups and preference of vowels were tested for significant differences. The results as indicated in Table 6.b, showed a statistically insignificant difference between the mean total congruent scores in preference of vowels between male and female participants, U = [8798], p = 0.59 as depicted in Figure 3.

Table 6b: Comparison of mean total congruent scores in preference of vowels between age groups <25 years and ≥25 years

Vowels	Mean (SD) Total congruent scores	p-value
<25 years	2.10 ± 1.005	0.59
≥25 years	2.18 ± 0.92	

Association between age groups and preference of consonants

An evaluation of influence of consonants between age groups, below 25 years and above 25 years was carried out for each question (Questions 11-13, 15, 22, 24, 27, 34). The congruent and incongruent scores were calculated for each of the two groups (Table 7.a). A chi-square test of independence was performed to examine the relation between age groups and the preference of consonants. The relation between these variables was statistically insignificant, $p > 0.05$.

Table 7a: Association between age groups and preference of consonants

Consonants	<25 years		≥25 years		p-value
	Congruent	Non-congruent	Congruent	Non-congruent	
Umam, Utap	143	67	56	31	0.53
Boub, Uomul	82	128	32	55	0.71
Innim, Ippipe	105	105	46	41	0.65
Ugug, Umum	104	106	42	45	0.84
Lolo	86	124	38	49	0.66
Moulu	104	106	46	41	0.59
Mille	132	78	51	36	0.49
Titi	140	70	58	29	0.99

Comparison of mean total congruent scores in preference of consonants between age groups <25 years and ≥25 years

The mean scores of the association between age groups and preference of consonants (Table 7.a) were tested for significant difference. As summarized in Table 7.b, there is no statistically significant difference between the mean total congruent scores in preference of vowels between age groups, U = [8957], p = 0.78, as depicted in Figure 3.

Table 7b: Comparison of mean total congruent scores in preference of consonants between age groups <25 years and ≥25 years

Consonants	Mean (SD) Total congruent scores	p-value
<25 years	4.26 ± 1.49	0.78
≥25 years	4.24 ± 1.27	

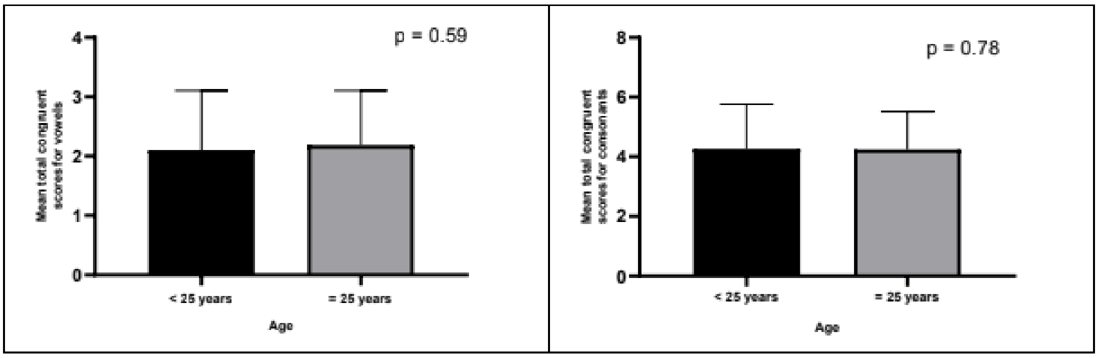

Figure 3: Association between age and preference of consonants/ age and preference of vowels among the survey sample

Association between age groups and preference of shape and word to a taste

Table 8a: Association between age groups and preference of shape and word to a taste in the study sample

Shape to taste	<25 years		≥25 years		p-value
	Congruent	Non-congruent	Congruent	Non-congruent	
Sweet or fatty taste	182	28	72	15	0.38
Sharp, acidic, citrusy taste	183	27	73	14	0.46
Crisp and fresh flavours and tastes	145	65	65	22	0.32
Word to taste					
Sharp, acidic and citrusy flavours	151	59	69	18	0.18
Sweet taste	142	68	63	24	0.41
Fatty taste	95	115	33	54	0.24
Crisp, fresh flavours and tastes	163	47	69	18	0.74
Sweet fatty taste	121	89	60	27	0.06

A chi-square test of independence was performed to examine the relation between age groups and the preference of shape to a taste and word to a taste (Table No. 8.b). The relation between these variables was statistically insignificant, $p > 0.05$. The results indicated that there was no statistically significant difference between the mean total congruent scores in preference of shape/word to a taste, between age groups, U = [8538], p = 0.36 (Figure 4).

Table 8b: Comparison of mean total congruent scores in preference of shape/word to a taste between age groups <25 years and ≥25 years

Shape/Word to a taste	Mean (SD) Total congruent scores	p-value
<25 years	5.62 ± 1.56	0.36
≥25 years	5.79 ± 1.46	

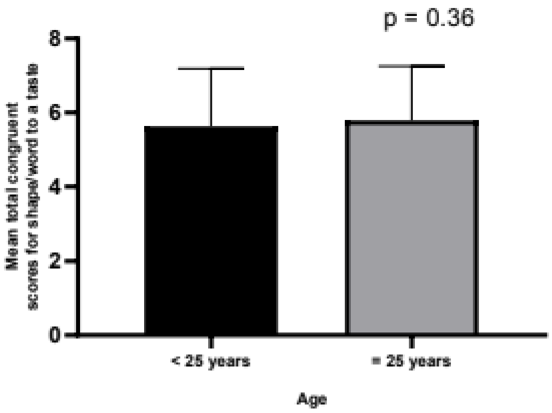

Figure 4: Mean total congruent scores for shape/word to a taste association with age

Association between sex and preference of shape and word to a taste

Table 9a: Association between sex and preference of shape and word to a taste in the study sample

Shape to taste	<25 years		≥25 years		p-value
	Congruent	Non-congruent	Congruent	Non-congruent	
Sweet or fatty taste	48	8	206	35	0.96
Sharp, acidic, citrusy taste	49	7	207	34	0.75
Crisp and fresh flavors and tastes	49	7	183	58	0.06
Word to taste					
Sharp, acidic and citrusy flavors	46	10	174	67	0.12
Sweet taste	32	24	173	68	0.03
Fatty taste	22	34	106	135	0.52
Crisp, fresh flavors and tastes	41	15	169	72	0.64
Sweet fatty taste	40	16	141	100	0.07

An attempt to explain the association of word to taste and shape to taste, was carried out (Questions 28-31, 35-38). The congruent and incongruent scores were calculated for each of the two groups (Table 9.a). A chi-square test of independence was performed to examine the relation between age groups and the preference of shape to a taste and word to a taste (Table 9.b). The relation between these variables was statistically insignificant (p>0.05), except for assigning the word 'Lumou' to a sweet taste (p = 0.03), wherein female participants (71.78%) were found to be more congruent than male participants (57.14%) (Figure 5).

Table 9b: Comparison of mean total congruent scores in preference of shape/word to a taste between male and female participants

Shape/Word to a taste	Mean (SD) Total congruent scores	p-value
Male (n = 56)	5.83 ± 1.35	0.50
Female (n = 241)	5.63 ± 1.57	

The results indicated that there was no statistically significant difference between the mean total congruent scores in preference of shape/word to a taste, between male and female participants, U = [6365], p = 0.50.

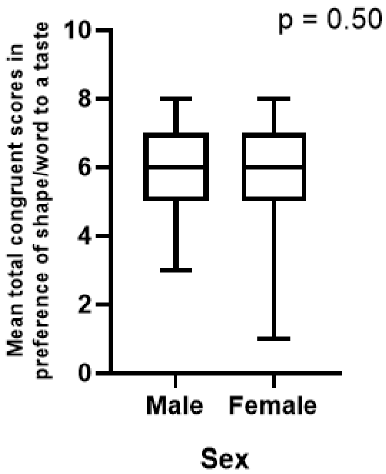

Figure 5: Mean total congruent scores in preference of shape/word to a taste associated with gender

Discussion:

Various attempts have been made to correlate the bouba-kiki phenomenon among differing age groups and in population subgroups. Previous studies have demonstrated cross-modal and multi-sensory perception evidence verifying the universal phenomenon 'Kiki-Bouba effect' (Ramachandarn and Hubbard, 2001; Spence, 2011). The objective of the present study was to investigate the presence of sound symbolism in the general Indian urban population and to assess the prevalence of the influence of vowels or consonants and whether the influence can be attributed to age/sex (Table 1). We further aimed to analyze the association between sound of words and visual stimulus, sounds of words and taste, and taste and visual stimulus. This study also assesses the influence of the consonant or the vowel on the responses. The influence of different factors such as age, gender, and education were also explored.

Our internet survey reached 299 participants out of which 2 of them were synesthetic. Hence, their response was excluded from analysis. As the form was circulated randomly via a social media platform (Whatsapp), there is a variation in the sample subgroup, as our sample consists of 56 males and 241 females (Table 2). The 297 people represented a mixed population speaking a variety of local or regional languages with the usage of a combination of Hindi, Marathi and English as the maximum used spoken language. This was followed by the usage of a combination of Hindi and English, and further practised by Hindi, Urdu and English among sects of population.. A representative graph has been given in Figure 6. No significant difference can be observed in the responses within linguistic groups, indicating the cross cultural robustness of the bouba-kiki effect. However, an internet based study conducted by (Woods *et al.*, 2013) did not yield any intra-modal associations of shape and color nor found anything parallel to a Kiki-Bouba distinction.

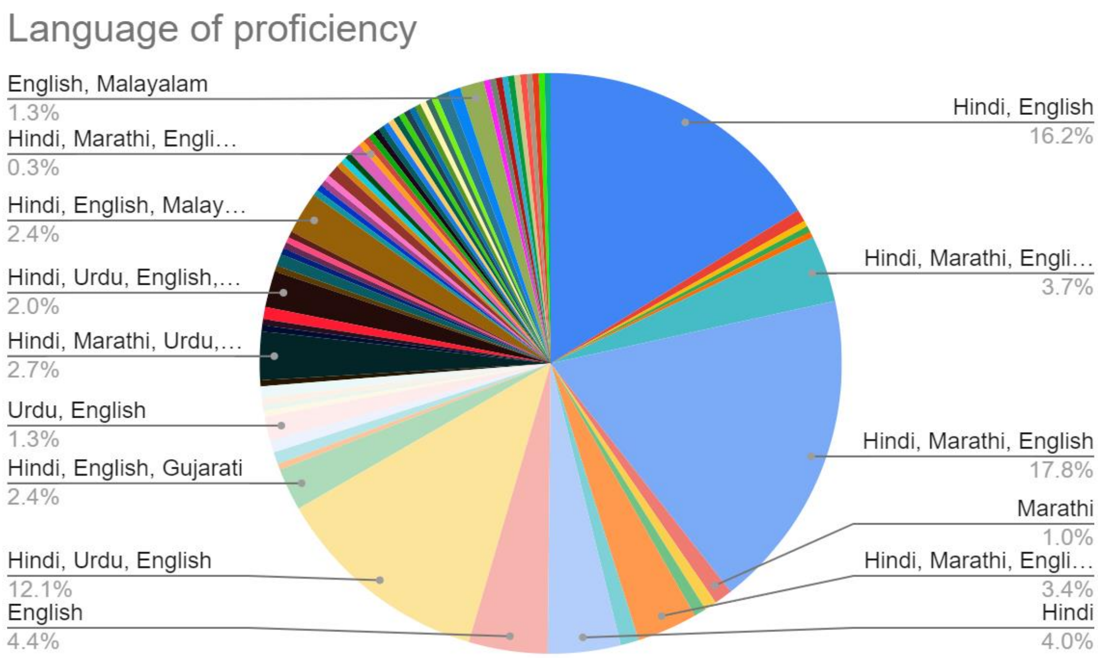

Figure 6: Linguistic details of participants

Our study further aimed to investigate the effect of consonants and vowels in the urban Indian population. For this purpose, each question was designed to investigate the effect of each vowel phoneme and each category of the consonants (plosives and sonorants). In this study, congruency is defined by round frames with sonorants (m,n) and spiky words with voiceless plosives (p,t). In question 2 (Table 3), there were 199 congruent responses out of 297 and 98 incongruent responses out of 297. And for similar kinds of questions (Q.3, Q.4, Q.6, Q.11;Table 2), the proportion of congruent and incongruent responses were nearly equal. There was a notable statistical difference between the mean total congruent scores and the mean total incongruent scores in the word-shape association task (p value < 2.2e-16 at 95% confidence interval). Even though multilinguistic , bouba kiki effect is prevalent in the population of Mumbai. The result agrees with the findings of (Ćwiek *et al.*, 2021) and their findings that across the globe the effect is rooted in a robust crossmodal correspondence between speech sounds and visual shapes. And our study augments the observation that myriad aspects of culture may play a mediating role yet there is a strong tendency for people across the globe to associate the spoken word bouba with a round shape and kiki with a spiky one.

Adeli and colleagues in 2014 reported that the congruence leads to a favorable affective evaluation and it also aids in cognitive tasks like classification evidence points to one principle in affective evaluations and cognitive tasks. Barton, 2016 further added that the congruence would therefore influence, in part, the daily social interactions. Our research indicates that congruence may have an impact on those residing in metropolitan areas who require intricate relationships to get about in their day to day life. Further studies on factors with respect to affect and congruence~~,~~ as well as the clock-work behind complex social behaviors may lend some clarity on how people make rapid decisions about their preferences.

Influence of consonants and vowels

Our findings suggest that vowels have a significant influence on the bouba kiki association as compared to consonants in the sample under consideration as depicted in Figure 1 (47.81% vs 1.68%). In case of pseudowords differing on consonants (e.g Utap/Umam) as per t test (p value = 0.4257), no statistically significant difference was observed between the association. However, pseudowords differing on vowels (Tutu/titi) a significant difference can be observed between the association as per the t test results (p value = 0.001074). This is also evident in case of incongruent pseudoword combinations (Tutu/Lili), as the association of round figures with Tutu is significantly greater than with Lili. For pseudowords differing on both consonants and vowels in congruent combination (e.g Bouba/Kiki), significant difference can be seen with respect to the associations as summarized in Figure 1. Thus, considering the Indian population, we suggest a higher influence of vowels in the word-shape association.

This influence can be attributed to the shape of the lips that assumes a round shape when pronouncing vowels like “o” and angular shape while pronouncing the vowels like “i”. (Passi and Arun, 2022)

The pseudowords were created by crossing either two consonant pairs with a wide range of vowels or two vowel pairs with a wide range of consonants (Table 3). Our results suggest that the influence of vowels in word-shape association is stronger than corresponding consonants. This phenomenon that we observed in the Indian population sample is different to the earlier reports (Fort et al, 2015). Previous reports indicate that consonants have a greater influence on the bouba kiki effect. This was apparently observed in case of French participants who were asked to match auditorily presented pseudowords with one of two visually presented shapes, one round and one spiky.

The influence of Consonants and Vowels associated with Gender (Male and Female):

The present study attempted to correlate the response of association tasks with sex to investigate the prevalence of the Bouba kiki effect in the male and female gender. (Table 4.a, 5.a). We calculated the scores of each individual evaluated on the basis of established literature. By conducting a Chi square test and Mann Whitney U test, we observed a statistically significant difference in the total congruent scores in preference of vowels between male and female participants where $p = 0.046$ at 95% confidence interval (Table 4.b) but not in case of preference for consonants where $p = 0.36$ at 95% confidence interval (Table 5.b). The comparative results are represented in Figure 2.'The association with regards to the Bouba kiki effect based on gender has not been sufficiently explored. Association of preference of vowels with males (Table 2) indicates that the male gender has a greater tendency to associate the words with shape under the influence of vowels rather than consonants. A similar study conducted by (Sidhu & Pexman, 2017) found that the participants showed preference for the round silhouette when presented with a round-sounding name, as compared to when they were presented the same with a sharp-sounding name. Further it was reported that the participants were found to choose the silhouette which was rounder, when shown a female name than when presented with a male name. Hence, the influence in case of males has to be studied in detail by specific tests involving both written stimuli and auditory stimuli to make any concluding statement regarding this.

We also investigated the difference in the influence of vowels and consonants in the prevalence of the Bouba kiki effect between different age groups. However, it was interesting to note that we found no statistically significant difference between the responses of the age groups (less than 25 years and more than 25 years old) as depicted in Figure 3. Furthermore, we observed that the mean of the total congruent scores and mean of the total incongruent scores in the taste - word/shape association task showed a statistically significant difference ($t = 18.832$, p-value $< 2.2e-16$) between the mean total congruent scores and the mean total incongruent scores of the taste - word/shape association.

An attempt was made to decipher if any association exists between age groups and the preference of shape to a taste and word to a taste (Table 8.a). Majorly it did not yield any significant correlation. A chi square test of independence was carried out on this data. The relation between these variables was found to be statistically insignificant (Table 8.b, Figure 4).

An attempt to gauge any association of word to taste and shape to taste (Table 9.a)~~,~~ yielded results which were statistically insignificant ($p>0.05$) (Table 9.b). However, there was a relation between 'Lumou' to a sweet taste ($p = 0.03$), wherein female participants (71.78%) were found to be more congruent than male participants (57.14%) (Figure 5). The results published by a previous study showed that subjects preferred to map symmetrical, Bouba shapes with fewer protrusions as pleasant and sweet; and higher protrusions in asymmetrical Kiki shapes as unpleasant and sour (Salgado-Montejo *et al.,* 2015).

As the study was an internet-based study carried out during the pandemic, the responses couldn't be timed to calculate speed of the response. Also, as there was no auditory stimulus provided to the subject, the effect observed cannot be attributed to only audio-visual or visuo-visual. Additionally, it needs to be checked whether this influence can also be observed in case of auditory stimuli where no written words are presented. Most of the population sample was literate enough to read the questionnaire that was in English language, except a few sample points (pre-school and younger individuals).

To conclude, the bouba-kiki effect seems to be the most composite phenomenon. Ever since its discovery to its being ambiguous to point to a set of features as being responsible for this effect. The bouba-kiki effect could be influenced by a host of factors, including acoustic, articulatory, and phonological properties of the speech stimuli (Ćwiek *et al.,* 2021; Hung *et al.,* 2017; Shukla, 2016). Based on our analysis, we conclude that the effect of Sound symbolism is prevalent in the general population. Notwithstanding these limitations, our research offers the most compelling evidence of the bouba-kiki effect's prevalence among Mumbai's Indian population—a multicultural community—to date. Broader applications of this research range from devising sensory substitution remedies for learning problems to neuromarketing. Further research is needed to systematically test the possible influences of these different factors on the bouba-kiki effect.

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USE OF VERMICOMPOST IN LAND RECLAMATION

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The organic wastes and residues offer the best possible means of restoring the productivity of severely eroded agricultural soils or of reclaiming marginal soils. The proper use of organic amendments is utmost important in maintaining the soil moisture level and hence the fertility and the productivity of the soils and in minimizing the wind and water erosion. The desired increase of water holding capacity will improve the ability to supply nutrients to the soil. Nowadays, the cultivable lands are gradually becoming the sites for constructing houses and industries. Due to the emergence of population, we need more cultivable lands. Reclamation of soils without environmental pollution is the urgent need of the hour.

Land reclamation refers to the process of converting degraded, damaged, or abandoned lands into areas that are useful and productive. It involves restoring the functionality and ecological health of the land to make it suitable for various purposes, such as agriculture, forestry, urban development, or conservation. Land reclamation aims to reverse the adverse effects of human activities, natural disasters, or industrial processes on the land. Earthworms in land reclamation, focusing on their significance in the context of metal-contaminated soils. The rapid industrialization of modern society has significantly impacted soil environments, detrimentally affecting earthworm populations. However, earthworms can serve as potent agents for reclaiming lands rendered derelict due to human activities. Beyond being valuable indicators in soil Ecotoxicological assessments, earthworms contribute substantially to terrestrial ecosystems by enhancing soil physical structure, fertility, and plant growth. Depending on the intended use of the reclaimed land, infrastructure development may be necessary. This could include constructing irrigation systems, roads, and other facilities to support agricultural, residential, or industrial activities. In cases where the land is contaminated due to industrial activities or pollution, land reclamation includes remediation efforts to remove or mitigate harmful contaminants. This may involve soil remediation technologies and the introduction of soil-enriching agents. For the successful reclamation of metal-contaminated soils, the improvement of unfavorable physical and chemical conditions is imperative to facilitate plant establishment. The inoculation of earthworms into such soils emerges as a transformative strategy, accelerating plant growth, increasing nutrient and metal bioavailability, and enhancing metal uptake by plants.

The effects of earthworms on metal speciation and bioavailability, emphasizing trials of earthworm inoculation for the reclamation of Pb/Zn mine tailings in Lechang, China. Earthworms, deemed suitable bioindicators of soil chemical contamination, offer an accessible and economical means of assessment. The use of earthworm tissue analysis as an index for heavy metal bioavailability in soils is important. It highlights the earthworm toxicity test as a standard protocol for assessing chemical toxicity, though challenges persist in standardizing chronic toxicity tests to detect subtle, long -term effects on earthworms. Laboratory experiments showcase innovative applications, such as using earthworms to disperse bioaugmented PCB-degrading microorganisms, resulting in improved soil aeration, increased soil carbon and nitrogen content, and modified soil microbial communities. The inclusion of earthworms in plant-based remediation techniques not only enhances plant growth but also expedites the restoration of soil ecosystem function.

Vermicomposting is a process in which earthworms are used to convert organic materials into humus-like material known as vermicompost. A number of researchers throughout the world have found that the nutrient profile in vermicompost is generally higher than traditional compost. In fact, vermicompost can enhance soil fertility physically, chemically and biologically. Physically, vermicompost-treated soil has better aeration, porosity, bulk density and water retention. Chemical properties such as pH, electrical conductivity and organic matter content are also improved for better crop yield. The countless uses of vermicompost in agriculture, precisely the use of vermicompost in organic farming, is due to its ability to act as a soil conditioner and to improve the soil texture wholly. This creates a healthy environment in which the plants can thrive. They are majorly used as enhancers of

The use of vermicompost, a nutrient-rich organic fertilizer produced through the decomposition of organic matter by earthworms, represents a sustainable and eco -friendly approach to enhance soil fertility and plant growth. Vermicompost is a valuable end product of the decomposition process carried out by earthworms, transforming organi c waste into a nutrient-dense material with enhanced soil-conditioning properties.

Vermicompost contains important elements such as NPK, Carbon, Magnesium, and Calcium along with other micro and macronutrients that ameliorate soil fertility and help in improving plant growth. Also, vermicompost contains beneficial microbes that work symbiotically with the plant unlocking additional benefits for the plants. The increase in soil fertility is the primary reason explaining the uses of vermicompost in agriculture. Earthworms help in increasing the soil porosity which increases soil aeration leading to better soil productivity. They further improve the water retention capacity of soil thereby reducing wastage of water. Vermicompost harbors certain Nitrogen-fixing microbes that catalyze root nodule formations and symbiotic mycorrhizal associations, enriching the soil ecosystem.

The evolution of land reclamation strategies, moving from agriculture -focused to amenity and wildlife conservation, is explored. The chapter underscores the difficulties posed by heavy metal contamination in soils and the need for comprehensive understanding, especially concerning plant–soil–animal interactions, to maintain sustainable vegetation on such lands. Earthworms contribute to the biodiversity of the soil ecosystem. Their activities create a habitat for various microorganisms, insects, and other soil-dwelling organisms, promoting a balanced and resilient ecosystem. The complicated interaction of earthworm activities in influencing soil physico-chemical and biological processes, along with their impact on plant root growth, is discussed. The chapter reviews past experiences in utilizing earthworms for land reclamation, providing insights into their potential in addressing the challenges posed by heavy metal-contaminated soils. Successful land reclamation projects often involve engaging local communities. Community participation can provide valuable insights, ensure the acceptance of reclamation efforts, and contribute to the sustainable use of the reclaimed land. Land reclamation using earthworms aligns with sustainable and organic farming practices, reducing the reliance on chemical fertilizers and pesticides. This can lead to a more environmentally friendly and cost-effective approach to land restoration.

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