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Genome-editing opportunities to enhance cattle productivity in the tropics

Luiz Sergio de Almeida Camargo[1*†] and Jorge Fernando Pereira[2†]

Abstract

The livestock performance in tropical regions has been limited by environmental conditions that causes heat stress and favors the development of parasites and diseases, impairing animal health. Heat stress disturbs animal homeostasis and affects animal production and fertility, with negative impacts on meat and milk quality. Flies and ticks proliferate easily under hot-humid weather, which makes difficult the control of their population, resulting in an increased parasitism. Tropical pastures usually have high dry matter production, but it is challenging to keep high production and quality under different environmental conditions throughout the year, constraining animal performance. Several strategies have been adopted in an attempt to overcome such hurdles in the tropical regions, but definitive solutions are yet to be implemented. In the last 20 years, biotechnologies, such as in vitro embryo production and genomic selection, have played an important role on cattle production in tropical countries. Genome editing (GnEd) is the novel tool in the toolbox for cattle production. GnEd with genomic selection offers the opportunity to boost the genetic gain in breeding programs of tropical cattle in fewer generations. It can be applied for disease resistance, to control parasite population, and to improve pasture quality and tolerance to biotic and abiotic stresses, favoring animal health and nutrition. Moreover, there is a perspective for the use of GnEd to control cattle methane emission by editing genes of methanogens present in the rumen. Although GnEd can already be applied to improve some traits, studies are still required for the identification of candidate genes in animals, tropical pastures, parasites, and microorganisms that can be targeted by gene editing in order to offer a robust contribution to the improvement of cattle production in the hot regions. Some examples of the use of GnEd are presented in this review, focusing on new perspectives of using GnEd to increase cattle production under the challenges of the tropical environments.

Keywords: Livestock, Heat stress, Milk, Pasture, Gene modification

Introduction

The world population is projected to reach 9.7 billion people in 2050, with most of the population growth estimated for countries located in tropical or subtropical regions, as Africa and Asia (United Nations, Department of Economic and Social Affairs 2019). The demand of food for this growing population needs to be fulfilled without increasing the pressure on land usage. Thus, increased food productivity is mandatory. However, crop and animal production in Asian and Africans countries have been limited by several factors, being the environmental conditions critical for livestock, as most countries are located between the tropics of Cancer and Capricorn. High temperature and humidity in the tropics cause several impacts on animal production, health and welfare, reducing productivity (Oke et al. 2021). Hot-humid weather is also an issue for Latin American regions located in the tropical belt, where the climate is a barrier to increase productivity (Garcia et al. 2015).

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One of the main problems in the tropics is heat stress, which disturbs animal homeostasis affecting the metabolic and hormonal status. To face this challenge, the body has to make physiological adjustments that lead to an increase in heat loss and reduction of heat production (dos Santos et al. 2021). All those adjustments have a price that is usually associated with low fertility and performance on milk and meat production. Heat stress can also have a direct impact on cell viability, as seen in lymphocytes, oocytes and embryos when exposed in vitro to high temperatures and humidity (Camargo et al. 2019; Paula-Lopes et al. 2003). Unfortunately, heat stress is not the only problem for cattle in the tropics. Ectoparasites, diseases, pasture quality and production throughout the year are also issues that require much attention. Ticks and tick-borne diseases can be found in tropical region as well as horn and screwworm flies, resulting in economic losses (Grisi et al. 2014; Jongejan and Uilenberg 2004). Also, tropical forages have high capacity of dry matter production, but there are several traits needing improvements (Pereira et al. 2018; Simeão et al. 2021), which constrain animal performance.
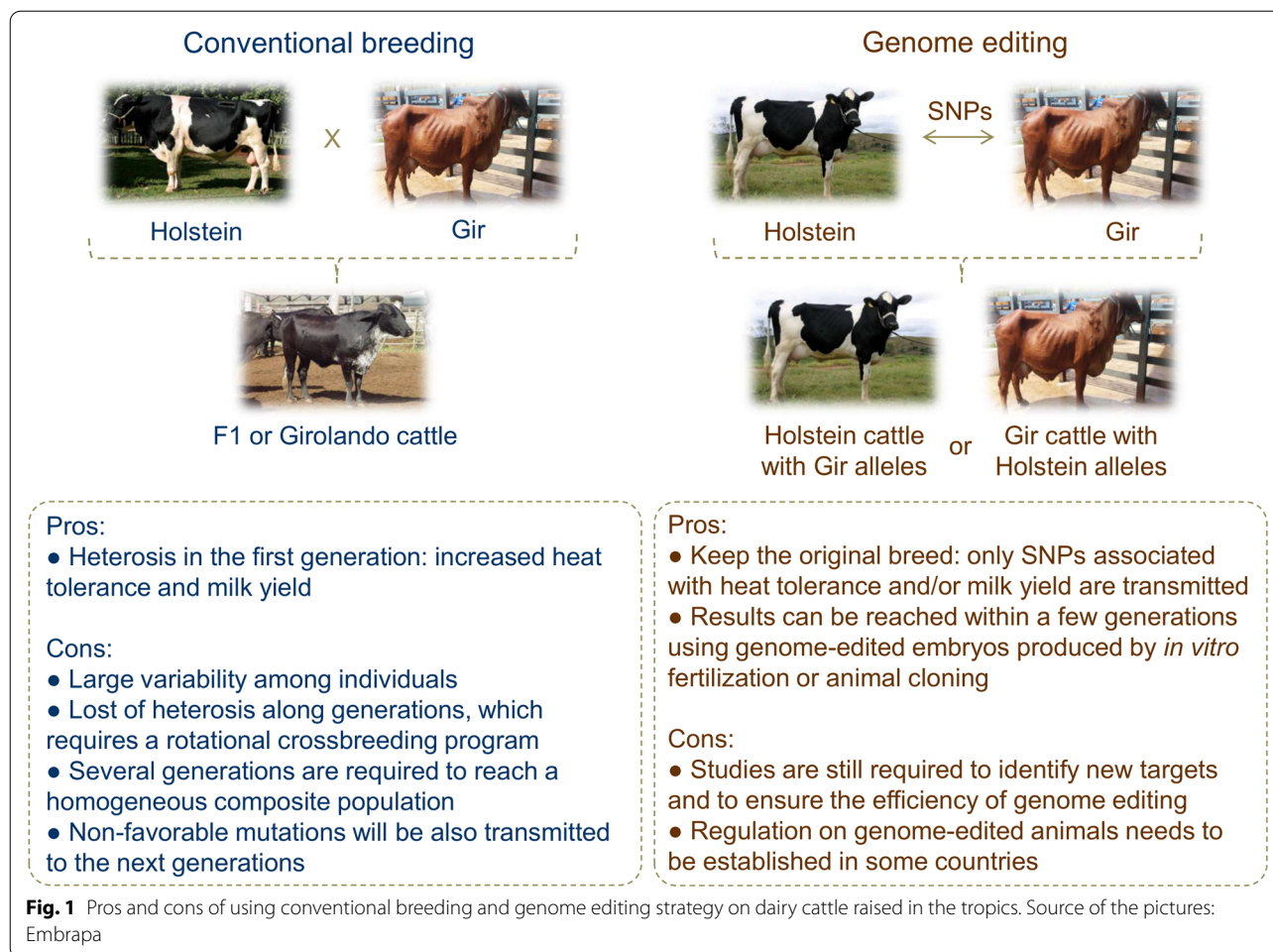
No single solution for livestock in the tropics is at hand. Capacity building, herd management, cooling strategies, vaccinations, and breeding programs are strategies that have being used with a range of success, depending of the country. In the last decades, biotechnologies have assumed an important role on cattle production. In vitro embryo production (IVP) and genomic selection have been applied with success (Panetto et al. 2020; Viana et al. 2018) in some tropical countries. The arising of genome editing (GnEd) opened new opportunities for cattle breeding as it can be used to fix target alleles for monogenic traits, delete undesirable recessive alleles and increase the frequency of favorable alleles for polygenic traits (Hickey et al. 2016). For instance, editing of 20 loci of a target trait plus genomic selection could double the genetic gain over 20 generations when compared with genomic selection alone (Jenko et al. 2015). Indeed, GnEd can offer possibilities of changes that would take generations to occur in cattle. Single or multiple alleles associated with a desirable trait in a specific breed can be moved (introgression) to another breed without crossbreeding. Moreover, if the allele or alleles are already in the target breed but at a low frequency, that can be increased in one or two generations because the editing can be performed in thousand zygotes from different cows and sires in a IVP laboratory. Such possibility, along with the simplicity and low cost, makes GnEd a powerful tool to cattle in the tropics and an alternative to conventional breeding (Fig. 1). For instance, alleles associated to a better body management of heat (heat tolerance) found in a tropical-adapted but low productive performance breed could be introduced to a heat-sensitive high productive performance breed. Frequency of alleles associated to tick tolerance could be increased or introduced into a given breed. The application of GnEd in the tropics is not only for animal breeding. Tropical pastures can be edited to increase drought tolerance and digestibility to provide good quality forages to feed animals along the year. Ticks and flies can be edited so that their population could be controlled in order to decrease the impact on animal health. Resistance to infectious diseases can also be created through changes in pathways from infection to the spread of the pathogen. Methanogen archaeas in the rumen can be edited to reduce methane production, minimizing the greenhouse effect caused by rumen gas emission. Thus, this review is focused on current status and perspectives for the use of genome editing tools to increase cattle productivity in tropical environments.

GnEd to address heat stress concerns

Heat stress is a major problem in the tropics and subtropics. It results in lower productivity that increases the demand for land along the years; one concern is that the situation can become worse in tropical lands with the climate change (Henry et al. 2018). A study estimated losses in milk production that could reach 1.7 billion/year in 2050 and 2.2 billion in 2080 due to global warming in North America (Mauger et al. 2015). The heat stress in the USA can lead to losses of US\$ 1.2 billion/year with a reduction in milk production, especially in the southern states, when compared to a stress-free environment (Key and Sneeringer 2014). In fact, climate change may contribute to further accentuate the effect of temperature on reducing the productivity of cattle herds (Nardone et al. 2010; Thornton et al. 2009), especially in tropical regions already affected by excessive heat and that hold a large bovine population. The impact of global changes on milk production in some Brazilian states can lead to an increase in heat stress that would be intensified in both warmer and colder months, and in the worst-case scenario, milk production could be limited to low productivity animals due to excessive heat (Silva et al. 2010). It is estimated that every 1 °C of temperature rise above thermal comfort may represent a reduction of 1.15 kg of milk/day in a warm climate region (Hayhoe et al. 2004). In addition to lower performance, heat stress can have effects on milk and meat quality (Summer et al. 2019; Zhang et al. 2020). Heat stress can also decrease productivity by decreasing reproductive efficiency, characterized by lower gamete and embryo viability, reduced conception rates and increased calving interval (Hansen 2009; Rensis and Scaramuzzi 2003; Takahashi 2012).

The effects of heat stress are more evident in European cattle (*Bos taurus taurus*), such as the Holstein and

Fig. 1 Pros and cons of using conventional breeding and genome editing strategy on dairy cattle raised in the tropics. Source of the pictures: Embrapa

Angus breeds, than those of Asian origin (Hansen 2020; Paula-Lopes et al. 2003), such as Gir and Nellore breeds, also known as zebu breeds (*Bos taurus indicus*). However, *B. taurus indicus* cattle are less productive than *B. taurus taurus* (Ahmed and El Amin 1997; Guimarães et al. 2002). On the other hand, *B. taurus taurus* animals are more sensitive to heat stress, compromising reproductive and productive performances (Hansen 2015). One solution has been crossbreeding to take advantage of heterosis (Miranda and Freitas 2009). For instance, Holstein (H) × Gir (G) crossbred cows have been the main source of milk production in several regions of Brazil, forming the synthetic breed Girolando (Freitas et al. 2013), established as 5/8 H × G, also encompassing other grades of blood, from 1/4 to 7/8 H × G. However, a large genetic variability exists in Girolando cattle, with animals with various degrees of heat tolerance (Carvalheira et al. 2021). Thus, it will take several generations of Girolando cattle to reach a homogeneous population due to the long interval between generations. Furthermore, crossbreeding ends up not only transmitting genes and mutations

of desired traits, but also unwanted traits that sooner or later may arise and compromise animal performance or health.

An alternative to circumvent the problem of heat stress without crossing breeds is the GnEd technology. With this technology, polymorphisms associated with heat tolerance can be transferred from one breed to another without requiring crossbreeding. This is the case of the slick hair coat (*SLICK*) *locus* located in the prolactin hormone receptor (*PRLR*) gene. The SLICK haplotype identified in heat-adapted breeds found in Central and South America results in animals with short and fine hair and with a better regulation of body temperature (Huson et al. 2014; Olson et al. 2003). The *SLICK locus* is found in Senepol cattle (Mariasegaram et al. 2007), a synthetic breed better adapted to hot climate. In 15/16 Holstein × Senepol cows, the presence of the *SLICK locus* resulted in animals with thinner and shorter hair, minimizing the loss in milk production in the summer (Dikmen et al. 2014). A mutation (C > T) in exon 11 (SLICK2) of the *PRLR* gene was found in Limonero and Carora cattle

(Porto-Neto et al. 2018). The slick phenotype and mutation can also be found in Caracu cows, a European breed brought to Brazil from Portugal in the beginning of colonization and well-adapted to subtropical climate (Camargo; personal communication). Slick mutation can be introduced in the *PRLR* gene of thermo-sensitive breeds to reduce heat stress when animals are exposed to high temperatures and humidity (Hansen 2020).

Other possibility to alleviate heat stress is to reduce the effects of sunlight on heat production in the animal body. Black or dark coat colors absorb more solar radiation and increases the solar heat gain (Hillman et al. 2001; Walsberg 1988). Red-and-white Holstein cows have lower rectal temperatures than black-and-white Holstein cows in the hot season (Isola et al. 2020). Also in the hot season, Holstein cows with coats predominantly black yielded less milk than those with coats predominantly white (Anzures-Olvera et al. 2019). Thus, reducing the size of black spots or diluting the black color on the coat of dairy cows can be an alternative to reduce the effects of heat stress on animal performance. A deletion of three base pairs in the pre-melanossomal protein 17 gene is found in diluted-colored Highland cattle (Schmutz and Dreger 2013). By the introgression of that mutation in Holstein embryos, it was possible to dilute the black color in a calf, producing a grey-and-white animal (Laible et al. 2020).

Although those two examples can already be targeted by GnEd, they probably are not sufficient to address the effects of heat stress. New alleles associated with heat tolerance need to be identified in order to be targeted by GnEd. For that, genome-wide association studies (GWAS) with fine-mapping analysis using distinct cattle populations are required to identify genomic regions that could be associated with the regulation of body temperature. For example, *B. taurus indicus* cattle are known to be more heat tolerant than *B. taurus taurus* cattle, and such distinction may be related to differences not only in production performance between breeds, but also in physiological adaptations that contribute to the regulation of heat gain and loss in *B. taurus indicus* cows (Hansen 2020). Recently, a GWAS was performed in Brazil using information from Girolando cattle aiming to identify genomic regions that could be associated with variations in vaginal temperature during hot-humid seasons (Camargo, personal communication). More than 35,000 data points of vaginal temperature were recorded within 48 h from 763 Girolando cows during the summer, and GWAS was performed using random regression models for vaginal temperature, with gene ontology (GO) enrichment analysis being performed by Cytoscape software. Genetic variance over 0.4 was found in chromosome 15, 17 and 23 in a 10-consecutive SNPs window. Four genes with biological functions on adaptive thermogenesis were identified in chromosome 15: uncoupling protein 2 (*UCP2*) and 3 (*UCP3*), caseinolytic mitochondrial matrix peptidase chaperone subunit B (*CLPB*), and phosphodiesterase 2A (*PDE2A*). GO analysis revealed relevant enriched pathways as a response to superoxide (*UCP3*) and to oxygen radical (*UCP3*), mitochondrial transport (*UCP2, UCP3* and *PDE2A*), response to temperature stimulus (*CLPB, UCP2* and *UCP3*), and response to heat (*CLPB*) and cold (*UCP2* and *UCP3*). Even though preliminary studies and a fine-mapping analysis are still required, such data suggest that causal mutations associated with the regulation of body temperature can be found in other candidate genes and, once the function is well-established, they can be potentially targeted by GnEd.

GnEd to increase quality and milk yield in tropical cattle breeds

Milk is one of the main food sources for people living in the tropics. It has not only a nutritional importance, but also an economic and social significance for large and smallholders. While large famers need to produce milk efficiently in order to make the farming activity profitable, smallholders can milk cows to feed the family but also sell the excess of milk to dairy companies for some revenue. Both cases need cows producing milk with a reasonable efficiency in a hot weather. Unfortunately, most of *Bos taurus indicus* breeds do not produce milk efficiently (Ahmed and El Amin 1997; Guimarães et al. 2002). Among several reasons, and compared with *Bos taurus taurus,* the lack of genetic selection appears to stand out as one of the main reasons for low productivity. For instance, the genetic breeding program for Brazilian Gir dairy cattle started only in 1985, and despite the contribution in the increase in milk yield (Panetto et al. 2020), the productivity is still lower than in European dairy breeds.

Traditional genetic selection programs based on progeny tests take long to show robust results due to long generational intervals and low intensity of selection. Although genomic selection has been used in genetic breeding programs for some *Bos taurus indicus* breeds (Panetto et al. 2020), there is now a chance to enhance genetic improvement in tropical cattle. The GnEd offers the opportunity to introduce SNPs associated with milk yield in the genome of thousands of embryos in just one generation. Being a polygenic trait, increasing milk yield or quality using GnEd will not be an easy task and will depend on the identification of SNPs using GWAS and fine-mapping studies. One issue may be the introduction of multiple SNPs in the genome of preimplantation embryos. Although this has not been tested in cattle yet,

it is possible to introduce multiples site-specific mutations in the genome using genome editing (Cong et al. 2013; McCarty et al. 2020; Sansbury et al. 2019).

While multiplex genome editing is no feasible in cattle, GnEd can be used to introduced specific single-mutations associated to milk yield in the genome of tropical cattle. A mutation that results in an amino acid change (phenylalanine > tyrosine) in exon 8 of the growth hormone receptor (*GHR*) gene of Holstein, Jersey and Ayrshire breeds is associated with an increased milk yield (Blott et al. 2003; Rahmatalla et al. 2011; Viitala et al. 2006). One of the roles of growth hormone (GH) is the stimulation of milk and protein production, which can occur indirectly through systemic changes, such as in food intake, blood flow and nutrient sharing for the mammary gland (Bauman 1999), but may also involve direct mechanisms in the epithelial cells of the mammary gland (Svennersten-Sjaunja and Olsson 2005). Receptors of GH have been identified in the mammary gland (Plath-Gabler et al. 2001), and an in vitro study with bovine mammary gland cells showed that GH can stimulate the expression of several genes of major milk proteins, such as caseins and alpha- lactalbumin and influence milk production (Zhou et al. 2008), which indicates the functionality of the GHR. This same specific mutation has not been reported for *Bos taurus indicus* cattle (El-Nahas 2018; Ramesha et al. 2016) and thus, it is a potential target for editing in order to contribute to milk production in those breeds.

Another possible target is a point mutation in the stearoyl-coenzyme A desaturase (*SCD1*) gene. This enzyme is responsible for the endogenous synthesis of conjugated linoleic acid (CLA) C18: 2 cis9, trans11 in the milk fat of lactating cows (Corl et al. 2001). CLA has been reported to be beneficial to human health, showing anti-carcinogenic, anti-obese and anti-hypertensive properties (Dilzer and Park 2012; Koba and Yanagita 2014; Ringseis and Eder 2009). A single nucleotide modification in the *SCD1* gene results in a non-synonymous codon change leading to an amino acid change (A > V) in the enzyme, causing an increased ratio of CLA without affecting the index of unsaturated fat in milk in Holstein cows (Schennink et al. 2008). The frequency of variant V in the Holstein cattle population was found to be low in herds of Netherlands (6.7%), (Schennink et al. 2008) and Canada (13.6%) (Kgwatalala et al. 2007). In the Jersey breed, a low frequency (5.2%) for variant V was also reported (Kgwatalala et al. 2007). In a study with a limited number of Gir cows, some SNPs were found, but none of them with the modification that results in the variant V (Freitas et al. 2013), suggesting that its frequency in Gir cattle is also low. In this case, GnEd can be used to increase the proportion of CLA in milk fat in tropical dairy cows,

benefiting consumers and aggregating value to the dairy products. Certainly, increase of milk yield and quality in tropics with GnEd will not be possible if animals are not appropriated fed. In the tropical regions cattle are mainly raised on pastures and those need to efficiently feed the animal all over the year.

GnEd to control tropical parasites

Tropical regions have as a feature an abundance of flies, mosquitos and ticks that benefit from the high humidity and hot weather to proliferate. Some of those ectoparasites can cause a direct effect on the animal, debilitating its health with consequences on growth, weight gain and milk yield, decreasing productivity (Rashid et al. 2019). One of those pests is the New World screwworm (*Cochliomyia hominivorax*) that infests the Caribbean and South American regions, with the larvae causing primary myiasis in cattle, although it can also parasite other warm-blood animals (Costa-Júnior et al. 2019). Screwworms were eradicated from USA and Central America using the sterile insect technique (SIT), where male sterile flies are released systematically to the environment to compete with native fertile males (Wyss 2006). The sterilization is achieved by radiation of pupae and require a mass production of quality insects to be sterilized (Scott et al. 2017). Although it was successful, the eradication screwworm program is expensive and challenging as it requires a mass production of male flies. The GnEd offers an opportunity to develop new pest control technologies that could be associated to SIT program. By disrupting the *transformer (tra)* gene, which controls sex determination in *C. hominovorax*, it was possible to knockout the gene to induce masculinization of surviving XX flies. With that, for instance, it will be possible to use GnEd to produce only males for the *C. hominovorax* SIT program (Paulo et al. 2019), which can contribute to reduce cost and to establish an effective program in South America.

Tick and tick-borne diseases are among the major constraint to livestock production in tropical and subtropical regions. While ticks can cause irritation, allergy and other toxic conditions that debilitate the organism, babesiosis and anaplasmoses, two of pathogens transmitted by ticks, increase the morbidity and mortality (Jongejan and Uilenberg 2004). A study with beef cattle detected potential candidate genes and significant SNPs associated with low tick infestation (Mota et al. 2018). A GWAS with a F2 population of Holstein x Gir cattle identified genes involved in the immunological functions, as *Triggering receptor expressed on myeloid cells* (*TREM*) *1* and *2* and *Cluster of differentiation 83* (*CD83*) genes that were associated to tick resistance. Most of animals resistant to tick infestation presented both maternal and paternal alleles originated from Gir breed (Otto et al. 2018).

Those sorts of studies are helping to pave the road toward the identification of causative mutations that can be targeted by GnEd in a near future. On the other hand, GnEd can be applied to control tick population, as shown in flies (Paulo et al. 2019) and mosquitos (Feng et al. 2021; Macias et al. 2020), by manipulating the tick genome in order to reduce population and capacity of infestation (de la Fuente 2018). An approach called Receptor-Mediated Ovary Transduction of Cargo (ReMOT Control) was developed to genetically manipulate mosquitoes in order to overcome the need of embryo injection and it can be extended to other invertebrates (Chaverra-Rodriguez et al. 2018), making the GnEd process easier and less costly in those species.

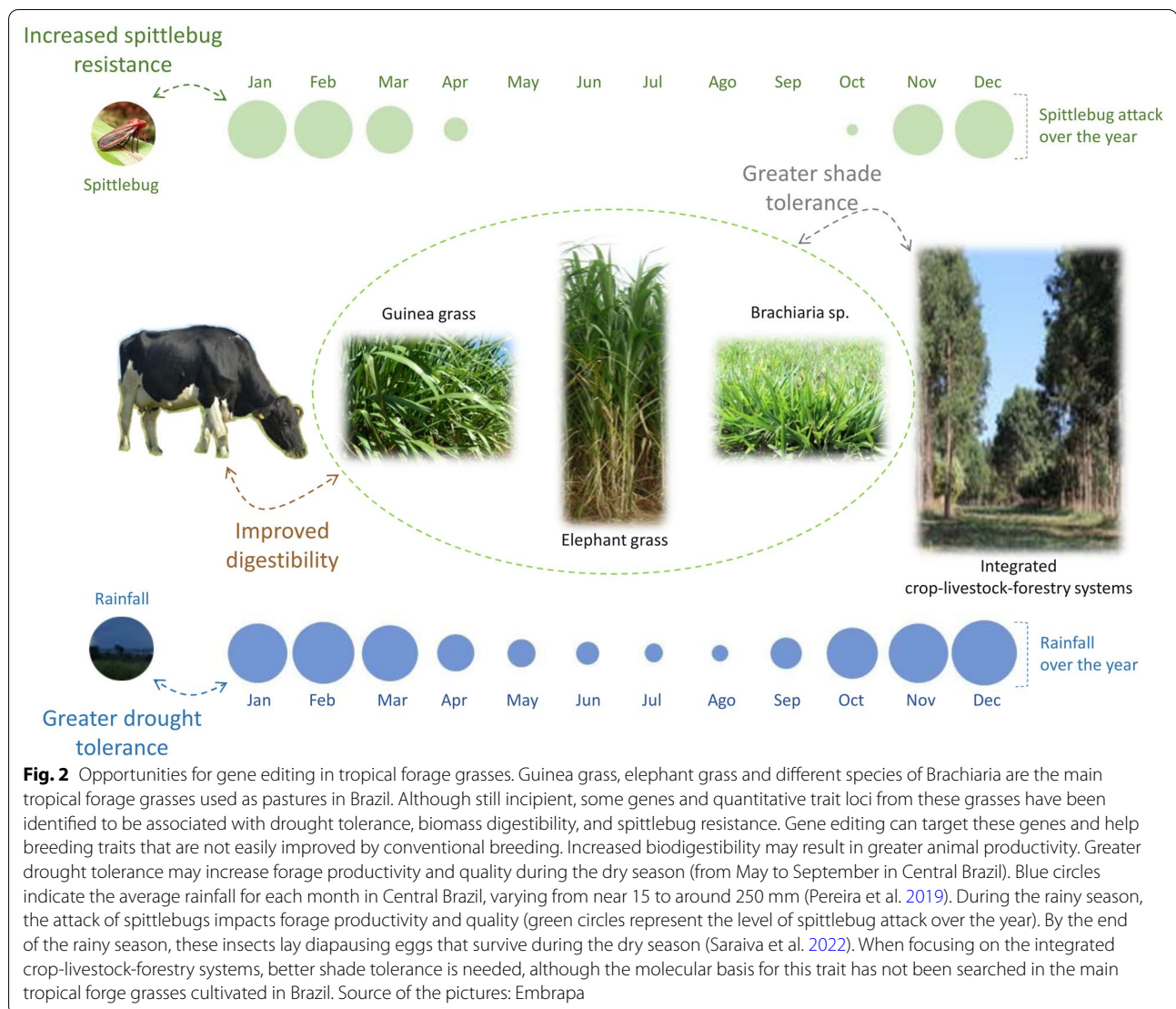### GnEd to improve the production and quality of tropical pastures

The feeding system influences the quality of meat, milk, and other dairy products (Delgado-Pertíñez and Horcada 2021; Moscovici Joubran et al. 2021). In a number of countries, most cattle are raised on pasture-based systems, which are viewed as more environmentally and animal welfare friendly (Moscovici Joubran et al. 2021). That is not different in tropical countries, where grazing is the most common way of cattle production. Environmental conditions in the tropics are not usually fit for temperate grasses and tropical forage grasses from different genera are grown as pastures, including Guinea grass (*Megathyrsus maximus* syn. *Panicum maximum*), elephant grass (*Cenchrus purpureus* syn. *Pennisetum purpureum*) and species of *Urochloa* (syn. *Brachiaria*). In Brazil, near 80% of the pastureland is cultivated with *Urochola* spp. and *M. maximus* represents around 10% of the cultivated pasture (Jank et al. 2014). In the past few years, cultivars of elephant grass are being well accepted by farmers, with one cultivar (cv BRS Capiaçu) cultivated in over 700 thousand ha only in four years (EMBRAPA 2020). Even though these tropical forage grasses represent a significant proportion of the pastures in the tropical region, there are research priorities and breeding goals that are not easily achieved by conventional breeding (Pereira et al. 2018; Simeão et al. 2021). There are opportunities for GnEd studies in tropical forage grasses to improve traits as digestibility, drought tolerance, spittlebug resistance, and shade tolerance (Fig. 2).

Digestibility can be defined as the proportion of forage biomass that can be digested and adsorbed by the animal. Biomass digestibility is dependent upon several components including cellulose, lignin, hemicellulose, and cell wall proteins (Pauly and Keegstra 2016). There have been efforts in order to elucidate the molecular mechanisms associated with biomass digestibility in tropical forage grasses. Two markers and candidate genes involved in the biosynthesis of cell wall molecules were found to be associated with digestibility in elephant grass (Rocha et al. 2019). Also in elephant grass, 39 markers were associated with eight feed quality traits, including acid detergent lignin and in vitro organic matter digestibility (Muktar et al. 2022). In *U. ruziziensis*, three genes associated with cell wall components were found to be highly confident loss-of-function alleles that may confer increased digestibility (Hanley et al. 2021). After confirming the role of such genes, GnEd may target them to improve the digestibility of tropical forage grasses.

Drought tolerance is the ability to sustain biomass or grain production under limited amount of water. This is an important trait because tropical forage grasses are perennial plants that eventually will face periods of drought under field conditions (Fig. 2). Cultivars that are able to keep higher forage production and quality under limited water availability are desirable. There is genetic variation for drought tolerance among tropical forage grasses and the molecular basis for that variation has been investigated. For instance, genes associated with cell wall metabolism may be important components of the drought tolerance in Brachiaria (Jones et al. 2021). In elephant grass, the dry matter produced per litre of water was used to evaluate water use efficiency (WUE) across 84 genetically diverse genotypes (Muktar et al. 2022). WUE was strongly correlated (>0.97) with total fresh weight and total dry weight, and several molecular markers associated with these traits have been found in several chromosomes (Muktar et al. 2022). For *U. brizantha*, the mechanisms of adaptation to water stress seems to be osmoregulation and deep roots (Santos et al. 2013). Since a large proportion of the soils in the tropics have acidic subsoils where aluminium (Al) stress is a major problem, the use of Al tolerant tropical forage grasses may lead to deeper rooting. Some genes for Al tolerance are available for *U. decumbens* (Salgado et al. 2017). Based on the subsequent confirmation of the importance of these genes for drought tolerance, WUE and Al tolerance, GnEd may be used to improve drought tolerance in tropical forage grasses.

Other important traits that can be targeted by GnEd in tropical forage grasses are spittlebug resistance and shade tolerance. However, the molecular basis for these traits have been less investigated. Improving spittlebug resistance in tropical forage grasses may lead into consideration some genomic regions and candidate genes found in *U. decumbens* (Ferreira et al. 2019). It is because many tropical forage grasses are susceptible to spittlebugs. The attack of spittlebugs is virtually inexistent during the dry season and it impacts forage productivity and quality

Fig. 2 Opportunities for gene editing in tropical forage grasses. Guinea grass, elephant grass and different species of Brachiaria are the main tropical forage grasses used as pastures in Brazil. Although still incipient, some genes and quantitative trait loci from these grasses have been identified to be associated with drought tolerance, biomass digestibility, and spittlebug resistance. Gene editing can target these genes and help breeding traits that are not easily improved by conventional breeding. Increased biodigestibility may result in greater animal productivity. Greater drought tolerance may increase forage productivity and quality during the dry season (from May to September in Central Brazil). Blue circles indicate the average rainfall for each month in Central Brazil, varying from near 15 to around 250 mm (Pereira et al. 2019). During the rainy season, the attack of spittlebugs impacts forage productivity and quality (green circles represent the level of spittlebug attack over the year). By the end of the rainy season, these insects lay diapausing eggs that survive during the dry season (Saraiva et al. 2022). When focusing on the integrated crop-livestock-forestry systems, better shade tolerance is needed, although the molecular basis for this trait has not been searched in the main tropical forge grasses cultivated in Brazil. Source of the pictures: Embrapa

during the rainy season (Fig. 2). Increased shade tolerance is desired in tropical forage grasses targeted to be used in the integrated crop-livestock-forestry systems, as *U. ruziziensis*. In this system, forages are grown alongside crops and trees with economic and environmental benefits. However, when trees became tall, shadow may impact the growth of forages. Even though there is variation for shadow tolerance among species of tropical forage grasses (Deinum et al. 1996; Guenni et al. 2008), the molecular basis for that variation is unknow.

Although tropical forage grasses are very important for animal production in the tropics, most (if not all) species can be considered as 'orphan' plants since molecular information is still rare. The developing of GnEd systems in a nongenetic model plants is challenging (Shan et al. 2020). Major bottlenecks for GnEd in tropical forage grasses include the large and usually polyploid genomes

of these species whose sequences are not available for most of them (Simeão et al. 2021), and the inexistency or inefficiency of transformation protocols for a number of these species (Bellido et al. 2021). Thus, more scientific developments are needed for GnEd of tropical forage grasses be able to significantly contribute with improvements in animal production.

GnEd to reduce greenhouse gas emission by cattle

Enteric methane from ruminants has been shown to have a significant contribution to human global greenhouse gas emission (Beauchemin et al. 2020; Opio et al. 2013). As tropical and subtropical regions hold a large ruminant population (Gilbert et al. 2018), one concern is to increase cattle productivity with as low as possible effect on methane emission. Methane in the rumen is

Table 1 A summary of challenges found in cattle farming in tropical regions and potential contributions of genome editing

Challenges	Genome modifications	Expected contributions
High temperature and humidity	Introduction of SNPs in the prolactin receptor gene Deletions in the Pre-melanossomal protein 17 gene Introduction of heat tolerance SNPs of *Bos taurus indicus* in *B. taurus taurus* cattle	Decrease the heat stress in *Bos taurus taurus* cattle
Poor milk yield performance	Introduction of SNPs associated with milk yield	Increase the milk production in *B. taurus indicus* cattle
High ectoparasites infestations	Disruption of *transformer (tra)* gene in *C. hominovorax* Introduction of tick resistance SNPs of *B. taurus indicus* in *B. taurus taurus* cattle	Control of screwworm infestations Increase the tolerance to tick infestations in *B. taurus taurus* cattle
Emission of greenhouse gas	Disruption of methanogenic archaea genes associated with methane production in the rumem	Decrease the cattle methane emission

produced by methanogenic archaea as *Methanobrevibacter gottschalkii* and *Mbb. ruminantium,* among others (Patra et al. 2017). Those rumen methanogens produce methane by converting H_2 and CO_2 into CH_4 (Lyu et al. 2018). Although there are several attempts to mitigate the rumen methane emission, none is sustainable yet (Islam and Lee 2019).

The GnEd can offer a new opportunity to mitigate the CH_4 production in the rumen by manipulating the genome of methanogenic archaea. It was shown that is possible to delete genomic sequences of *Methanosarcina acetivorans* in order to generate frameshift mutations using Cas9-mediated genome editing and also possible to introduce multiple deletions (Nayak and Metcalf 2017). The genome sequence of *Mbb ruminantium* is available (Leahy et al. 2010) and the function of some predicted gene clusters were shown (Bharathi et al. 2020). Further studies with rumen methanogens are required and will be useful to provide information about genes that play role on CH_4 production in the rumen. Then, trough GnEd will be possible to target multiples candidate genes in order generate mutant methanogens archaea that produce less CH_4 and use them to populate the rumen.

Regulatory aspects

The regulation of GnEd animals is still in discussion in some countries while in others, as Brazil, Argentina and Japan, there is a regulatory policy that can consider GnEd animals that do not contain foreign DNA (i.e. DNA sequences that do not exist in the specie) as non-GMO after case-by-case evaluations (Hallerman et al. 2022). That means that in some countries GnEd animals generated without foreign DNA can be regulated as conventional animals after approval by the respective regulatory agencies while in others the regulation is still unclear. Such discrepancies in the regulatory policies among countries need to be addressed in order to allow a worldwide application and commercialization of GnEd cattle and the derived products.

Conclusion

GnEd is a genomic tool that can revolutionize cattle production. There are perspectives to apply this technology in order to increase the animal performance under the challenges imposed by the tropical environments (Table 1). The genome editing can be performed in the animal aiming to introduce or to increase the frequency of one or more favorable alleles in a given breed or it can be performed in the species surrounding it, as tropical pastures, parasites and microorganisms, aiming to feed the animal properly and to improve health and quality of life. However, to GnEd accomplish its tasks, several studies need to be carried out in order to identify and increase our knowledge about potential targets, taking into consideration the biodiversity found in a tropical climate. In addition, investigations of unintended genomic modifications are required after every gene editing to avoid further off-targets effects. For last, the regulation of GnEd animals can differ among countries, which can restrain international trade of products derived from GnEd cattle while a worldwide consonant regulatory policy is not established.

Authors' contributions
Both authors contributed equally to the manuscript. Both authors have read and approved the final manuscript.

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Declarations

Ethics approval and consent to participate
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Consent for publication

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Competing interests

The authors declare that they have no competing interest.

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