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ORIGINAL RESEARCH PAPER

Gene Editing Cattle for Enhancing Heat Tolerance: A Welfare Review of the “PRLR-SLICK Cattle” Case

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Abstract In March 2022 the US Food and Drug Administration (FDA) published a risk assessment of a recent animal gene editing proposal submitted by Acceligen™. The proposal concerned the possibility of changing the cattle genome to obtain a slicker, shorter hair coat. Using CRISPR-Cas9 it was possible to introduce an intentional genomic alteration (IGA) to the prolactin receptor gene (PRLR), thereby producing PRLR-SLICK cattle. The goal was to diminish heat stress in the cattle by enhancing their heat-tolerance. With regard to unintended alterations (i.e., off-target effects), the FDA stated that the IGA posed a low, but still present, risk to animal safety. The aim of this article is to present some initial insights into the welfare issues raised by PRLR-SLICK cattle by addressing the question: Do SLICK cattle have better welfare than non-SLICK cattle when exposed to heat stress? Two potential welfare concerns are examined. The first is pleiotropy, an issue that arises when one gene affects multiple traits. Given the pleiotropic nature of prolactin, it has been suggested that the IGA for SLICK cattle may also affect their hepatic and other functions. The second concern relates not primarily to direct effects on cattle health, but rather to the indirect risk that this more heat-tolerant animal would just be used in the livestock sector under farming conditions that are such that the net welfare improvement would be non-existent.

Keywords PRLR-SLICK Cattle · Gene Editing · FDA Risk Assessment · Heat Stress · Enhancement · Animal Welfare

Introduction

In March 2022 the US Food and Drug Administration (FDA)—the US agency handling public health in the regulation of biological, alimentary, and pharmaceutical products—published a risk assessment (henceforth the *Risk Assessment*) [1] setting out its conclusions on a recent application of gene editing applied to farm animals, specifically cattle, submitted by the company Acceligen™. It is the FDA’s duty to provide risk-based evaluations of intentional genomic alterations (IGAs) in organisms such as farm animals—to ensure that they are safe and consistent with the claims made for them by the developers [2]. The case concerned the possibility of modifying the cattle genome in order to obtain a coat with shorter, slicker hair. Using the

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CRISPR-Cas9 technique [1], an IGA was introduced in the prolactin receptor gene (PRLR gene) in the cattle genome. This was done to create a phenotype with a slicker, shorter coat. The case has come to be known as the “PRLR-SLICK cattle” case.

The goal of the genetic modification was to diminish heat stress in the cattle by enhancing their tolerance to heat. While noting the occurrence of unintended alterations (i.e., off-target effects) in cattle, the Risk Assessment stated that these were not expected to represent a major concern. The FDA concluded that the IGA posed a low risk to animal health and safety [1]. It is worth stressing that the Risk Assessment did not conclude that there are no risks. The FDA actually emphasises that a low-risk determination “is not a determination of ‘safety’ under the Federal Food, Drug, and Cosmetic Act, but is instead a determination that a product is low risk enough that it is not an FDA enforcement priority. If FDA becomes aware of new information about risk, it may revisit these decisions” [2].

Although the Risk Assessment also examined the potential risks to human beings and the environment, this article focuses only on cattle-centred issues. The FDA reported on these issues by discussing the risks to animal safety in two sections of the Risk Assessment: *Molecular characterization* and *Phenotypic data and animal safety/health*. The FDA evaluation was based on data and information submitted by Acceligen™ on trials the company had undertaken. However, the FDA independently analysed the whole genome sequencing (WGS) data provided by the company [1]. The Acceligen™ trials involved four individual animals upon which the IGA introduction was attempted. Among these, only three then exhibited the IGA. One of these died after the IGA had been introduced for reasons that were determined to be unconnected with the IGA. The FDA’s statements regarding animal safety therefore exclusively addressed the occurrence of unintended alterations in the two surviving calves with the IGA.

The aim of this article is to provide some initial insights into the welfare impacts of the IGA on PRLR-SLICK cattle. We begin, in Section "Can we talk about improving animal welfare within the current farming system?", by briefly addressing a possible critical argument—namely,

that we should not discuss animal welfare, including any improvements in it, as long as the. enhanced animals continue to be farmed in a system that keeps animals for production purposes. Section "Heat stress in cattle" describes the effects of heat stress on cattle and the problems associated with those effects. In Section "PRLR-SLICK Cattle: A possible solution?" we then look at the result achieved by Acceligen™, the current literature on SLICK variants that have already arisen spontaneously, and the extent to which the naturally occurring variants have been shown to overcome the difficulties posed by heat stress. Section "Animal safety and the FDA Risk Assessment" considers what was reported in the FDA Risk Assessment regarding the health and safety of the calves treated by Acceligen™. The final two sections of the article explore two potential welfare issues raised by PRLR-SLICK cattle. The first, which is the topic of Section "Concerns about pleiotropy", is the issue of pleiotropy [4, 5], where one gene affects multiple traits. It has been suggested that the SLICK variant may also affect hepatic function in cattle [6]. The second, turned to in Section "Improved heat tolerance and the livestock sector", is not about the direct effects of the genetic modification on cattle health, but rather the indirect risk that the more heat-tolerant PRLR-SLICK cattle would merely be farmed, in the livestock sector, under even warmer conditions than previously, so that in effect the potentially positive welfare effects are lost. A short conclusion is presented in Section "Conclusion".

The philosophical literature on the issue of animal welfare is broad and detailed. Indeed, several studies of the relationship between animal welfare and the agricultural sector [7, 8] and that between animal welfare and genetic technologies [9, 10] have been published. While recognising the significance of this work, this article will not provide any new general discussion regarding the issues it covers. What has already been stated is adequate for the purposes of this article, and for the forthcoming discussion.

This is so because our prime focus is on the prevention of adverse welfare effects which are widely recognised as such. In this article, it is important to note that the notion of “welfare” is intricately linked to the notion of “health”, as highlighted in the FDA Risk Assessment.

There is a wider discussion about the future of cattle production when it comes to handling global issues relating to the climate and biodiversity crises. This important discussion has been left out in this paper, where focus is only on animal welfare. However, for a policy discussion it may be relevant to link the issue of gene editing to the mentioned wider discussion.

Can We Talk About Improving Animal Welfare Within the Current Farming System?

The aim of this article is to discuss welfare effects using the cattle that come out of the Acceligen™ project. However, the context of the article is the world of modern-day cattle farming. Therefore, we think that before examining the PRLR-SLICK cattle case, it may be worth addressing a potential critical question: Is it ethically meaningful to discuss animal welfare within intensive agriculture?

For example, Arianna Ferrari is critical of debate over genetic interventions designed to diminish animal suffering "if the subjects of these actions do not have fundamental rights, such as the right to live and live free (and thus do not have a good life), and are bred, kept and killed for human purposes" ([3], p. 74). According to her, in the relevant genetic modification projects there would be no genuine concern for animal welfare, but only an efficiency-based calculation, made within the farming system, that assumes that the quality of products is better guaranteed when animals are "maintained with a certain level of 'fitness'" ([3], p. 73). Consequently, Ferrari concludes, the discussion of animal gene editing is not centred "around strategies for protecting non-human animals, but rather [is] a discussion on better strategies to cope with some negative effects on the animal phenotype caused by human exploitation" ([3], p. 75).

Does this mean that it is simply not worth discussing animal welfare as long as the discussion does not relate to an abolitionist approach? Unlike Ferrari we believe that solutions for reducing animal suffering can still be explored within the current system. This can happen without taking a stance for or against abolition as the ultimate goal. Debate should not, in our view, be conducted solely through comparisons with a hypothetical world. It can also legitimately focus on the world with commercial dairy cattle farming. As Marcus Schultz-Bergin has claimed, "if we assume the only alternative is the current system, then the current system minus at least some of the suffering seems a morally proper move" ([11], p. 107).

Recognising that the suffering experienced by animals is a serious problem, we shall therefore proceed with the focus of improving animal welfare within current food production systems. Here a proposal made by Adam Shriver is relevant [12]. He stated that "not enough people are willing to become vegetarian to completely eliminate the suffering caused by intensive factory farming" ([12], p. 119). This relates to a distinction between the current reality and a hypothetical (or ideal) one. In the current world, unlike the hypothetical one to which the abolitionist account refers, the cessation of any animal use by humans cannot be conceived as the only solution to the animal welfare problem because it is unachievable in the short term. Accordingly, we could propose a time-based argument that considers the distinction between the actual world and the hypothetical one. Thus, acknowledging suffering as a major concern, it is important to focus on suffering that can be currently overcome in a specific set of circumstances without requiring ideal transitions that are unattainable in the short term. Such an approach enables pragmatic discussion of small-scale solutions to improve animal welfare within the current farming system.

It could be argued that these short-term strategies may hinder the development of long-term solutions that are considered to have greater ethical value. This objection refers to the technological fix problem. Devolder has already presented a response to this objection [13]. The point is not to argue over whether the reduction of animal suffering is brought about out of genuine concern for animal welfare or production efficiency. It is merely a matter of asserting that, having acknowledged suffering as a major concern, we believe it is ethically conceivable to discuss whether strategies to diminish it are valid. It should be acknowledged that the prevention of suffering is a complex and multifaceted issue, and as such, this article cannot provide a comprehensive analysis. However, it is hoped that what is discussed here can contribute to the ongoing debate.

Now, we should try to understand whether the gene editing project developed by Acceligen™ would in fact improve cattle welfare.

Heat Stress in Cattle

Maintaining a specific body temperature is critical in mammals for body function and animals' survival.

Body temperature is regulated by controlling internal heat generation as well as by controlling the exchange of heat with the environment. This process is called homeostasis. The conditions under which this body-temperature balancing capacity, sufficient to regulate body temperature, occurs define the thermoneutral zone (TNZ) [14]. The lower end of the zone is determined by the ability to conserve and generate heat in low temperatures. The upper end is determined by the ability to reduce heat generation and dispose of excess heat. The TNZ is determined by factors such as temperature, humidity, water availability, wind, and solar irradiation. An index commonly used is the temperature-humidity index (THI). This is "a single value representing the combined effects of air temperature and humidity associated with the level of thermal stress" ([15], p. 1947). Humidity exhibits a "strong correlation with thermal stress in humans and other large mammals" ([16], p. 243), "because it affects the amount of latent heat loss" ([17], p. 113). Heat stress arises when the amount of heat gained is higher than the amount the animal can dispose of. Heat in cattle – like that in other animals – originates both internally from metabolic processes and from the environment. Animals dispose of heat by radiation, convection, evaporation, and conduction [14]. The TNZ differs with breed, age, and weight. For example, "the TNZ of a 1-month-old calf is between 13 to 25 °C, and the TNZ of a heifer with 0.8 kg daily gain is between 0 to 15 °C" [14], whereas a lactating dairy cow can be maintained within a temperature range from -0.5 to 20℃ [18].

The health impacts of heat stress are of the utmost concern. Cattle certainly are vulnerable to rising temperatures. When a cow is no longer able to regulate its body temperature as a result of excessive heat, with the onset of criticality at around 25–26℃ [19], it will begin to exhibit clear symptoms of heat stress. One warning signal indicating that a critical state has been reached is an increase in the number of breaths per minute (bpm) to the point at which the animal is panting. Under normal heat conditions, the average ventilation rate for an adult dairy cow is 40–60 bpm. If more than 10% of the cattle in a herd are panting at 100 bpm, an emergency situation is indicated [20]. With regard to physical appearance and behaviour, clear signs of heat stress also include open-mouth breathing with the tongue protruding, excessive drooling or foaming, an extended neck, gathering in groups, and standing restlessly, with reduced lying time and walking activity [20–22]. Conversely, in what the United States Department of Agriculture (USDA) defines as the last critical stage [21], the responses are reversed. The head is now down, drooling ceases, breathing becomes laboured and ventilation rate decreases, and individual animals may isolate from the herd.

A large part of the scientific literature on problems arising from heat stress in cattle focuses on downstream effects on food production. Since cattle are central to the agricultural economy, most of the interest is reserved for understanding how heat stress affects productivity. However, for the purposes of this article, we are going to consider mainly cattle welfare. It is worth mentioning, however, that there is a debate over the possibility of relating welfare assessments to observations on decreases in milk production [23].

What, then, are the effects on bovine welfare caused by excessive heat? Although it is still a disputed issue, reduced lying time, resulting in excessive standing time, may end up causing lameness [22], which is "widely regarded as a major welfare problem for dairy cows" ([23], p. 4103). Changes in lying behaviour may also impair the animal's endocrine and metabolic functions by affecting, for example, the hypothalamo-pituitary-adrenal (HPA) axis [24].

Another significant effect of heat stress is on the immune system. Long-term heat stress manifests in increased concentrations of cortisol. The consequences of this include a reduction in the release of antibodies, cytokines and chemokines, and impairment of cellular immune functions [25]. "Further, heat stress impedes both cell mediated and humoral immunity thereby altering the balance for effectively maintaining the immune functions in dairy cattle" ([25], p. 99). Extreme heat can cause inappetence, which can affect the cow's nutritional health [22]. Similarly, in some cases it may lead to thirst, and then to an exacerbation of the dehydration as well as to deterioration in the animal's mental state and cognition [22]. Research has demonstrated that heat stress can impair the animal's ability to process information from the environment, respond to it accordingly, and be flexible in modifying its behaviour [26]. However, the authors of this research state that these capabilities are crucial principally for those animals that survive in the wilderness and less so for livestock living in confined spaces [26].

Last, but not least, heat stress may cause death. "Older cows had a higher risk of death during heat waves, even if physiological and health status likely played a more important role in their mortality" ([27], p. 4578). Among the more serious events involving heat-related cow deaths is one that occurred in the U.S. state of Kansas. With 6.5 million cattle, Kansas is the third largest state in the U.S. by number of cows raised [28]. The Kansas Department of Health and Environment stated that in June 2022, over the course of just a few days, approximately 2,000 cows died as a result of temperatures reaching 100°F (37–38°C), compounded by high humidity levels and limited wind.

Thus, exposure to heat stress has major detrimental effects on cattle welfare. The problems here have grown as average global temperatures have increased in recent decades. According to temperature measurements over the period 1850–2023, 2023 was the warmest year ever recorded with an increase of 1.18 °C above the twentieth-century average and the months July to December each being the warmest on record [29]. The 10 warmest years on record are the years 2014–2023 [29]. Along with an increase in average temperatures, there is also a rise in the frequency of heatwaves, i.e., extreme heat events of varying duration and intensity [16, 30]. In addition to changes in their frequency, these extreme heat periods are also increasing in duration and intensity [31]. "Whether the mean temperature increases of the coming decades are within the range that can be tolerated or not by existing distributions of different genotypes of cattle in the tropics, is essentially unknown" [32].

Regarding cattle, North et al. [33] conducted a study on the correlation between future climate projections and the impact of heat stress on cattle mortality, fertility, and production. In particular, they estimated that "77% of cows are already exposed to climate conditions likely to cause heat stress for at least 30 days each year, with 20% of cattle (those in the tropics) exposed to heat stress conditions year-round" ([33], p. 5), and that by 2100 "these percentages are projected to increase to 90% of cows exposed for at least 30 days each year, and 34% experiencing year-round heat stress" ([33], p. 5).

PRLR-SLICK Cattle: A Possible Solution?

Problems arising from heat stress require implementation of appropriate remedies. Among the solutions adopted, probably the most elementary is the provision of shelter for the cattle, so that they can stand in the shade. Using fans is also essential in delivering an unceasing supply of fresh air, as is the use of sprinklers, which constantly keep the animal wet, allowing them to shed excess heat by evaporation [34].

However, genetics may be part of the solution as well. With the significant recent developments in genetics, it has become possible to draw on CRISPR-Cas9 technique in the search for novel solutions to problems affecting livestock. In this regard, one of the most discussed solutions is to increase heat tolerance in cattle. The PRLR-SLICK cattle developed by Acceligen™ were created with an IGA. "IGAs in animals are changes to an animal's genomic DNA produced using modern molecular technologies, which may include random or targeted DNA sequence changes including nucleotide insertions, substitutions, or deletions" [35]. The IGA was accomplished by injecting genome editing reagents into embryos in vitro. The embryos were then transferred to surrogate mothers [1]. The IGA in the prolactin receptor gene (PRLR) truncates the prolactin receptor protein [1], i.e., the C-terminal region of the protein involved in JAK2/STAT5 activation during prolactin signalling [36]. "One of the actions of prolactin is to inhibit hair growth" ([6], p. 2). Thus, the IGA appears to affect prolactin's work, resulting in cattle that possess a slicker coat of hair than normal. Moreover, the IGA has been shown to be inherited [1]. This means that it can be passed on to offspring through breeding with in a PRLR-SLICK cattle line.

However, SLICK variants did not first appear because of the application of CRISPR-Cas9. Similar variants occur naturally in tropical cattle breeds. Even the FDA Risk Assessment points out that: "The IGA is the equivalent to the naturally occurring slick mutations that occur in several breeds of conventionally raised cattle where they likely developed as an adaptation to being raised in tropical or subtropical environments" ([1], p. 1). A similar variant was originally identified in the Senepol breed on the Caribbean Island of St. Croix [37]. It has also already been possible to transmit this special trait through conventional breeding, as has been the case with the

Holstein breed [37]. Although the variant obtained via CRISPR-Cas9 differs in terms of its genomic sequence, it is similar in its phenotypic characteristics ([1], p. 3).

What benefits are there for cattle from a short and sleek (SLICK) coat of hair coat of the kind that follows from the genetic modification? Research – conducted on cattle with a naturally occurring SLICK variant, not calves with the IGA developed by Acceligen™ – demonstrated that SLICK cattle have an improved ability to cope with heat in comparison with their non-SLICK counterparts [37–39]. One test was carried out at the University of Florida and involved Holstein cattle. The testing took place on multiple occasions between 30 July and 5 August 2013 using different cows in different sessions. It "was conducted to determine whether the superior ability of slick-haired cows to regulate body temperature during heat stress was due to differences in surface temperature or sweating rate compared with cows without the slick-haired phenotype" ([37], p. 5510). By measuring rectal temperature, skin temperature, sweating rate and ventilation rate, the researchers were able to show that SLICK cattle had lower values for all four parameters than non-SLICK cattle [37]. Their results supported the conclusion that SLICK cattle are better able to cope with heat [39]. Similar research was conducted in Florida and California, in July of 2020, involving both SLICK and non-SLICK cattle [38]. "This study demonstrated that the presence of the SLICK1 allele results in lower body temperature in young Holstein cattle exposed to the subtropical heat conditions found in Florida" ([38], p. 9224). "Current explanations for the enhanced heat stress response of slick-haired heifers rely on a more efficient heat loss capacity" [40].

Although our article focuses on cattle welfare, it is also worth briefly mentioning the possible efficiency gains for the livestock sector. Evidence has been provided that the SLICK mutation has benefits in terms of food production, with SLICK cattle in warm climates displaying a smaller reduction in milk yield compared with their non-SLICK counterparts [37, 41]. So, there may be a win–win here for cattle welfare and production efficiency.

In conclusion, the steady rise in average temperatures due to climate change requires new solutions to the problems presented by heat stress and its effects on cattle. The SLICK phenotype may provide benefits in this regard, as it results in an increased ability to cope with heat stress and thus may help cattle to remain within their thermoneutral zone (or TNZ) ranges. It should again be noted that the research mentioned in this section, on the benefits of the SLICK phenotype, only involved cattle carrying a naturally occurring SLICK variant. It did not examine the IGA. However, based on the identification of similar phenotypic characteristics (although a not identical genotypic sequence), the FDA "determined we can reasonably expect the cattle with the IGA and slick phenotype to be more thermotolerant than their longer-haired counterparts during periods of heat stress" ([1], p. 6). Therefore, a cattle gene editing project aimed at increasing cattle tolerance to areas exposed to heat events would seem to achieve its purposes.

Animal Safety and the FDA Risk Assessment

Although reports refer to PRLR-SLICK cattle, the IGA's established name is "SLICK alteration disrupting *Bos taurus* g.(NC_037347.1) fs(39,099,129–39099368) in exon 9 of the PRLR gene in *Bos taurus*" ([1], p. 1). The review conducted by the FDA determined that products resulting from Acceligen™ gene editing of bovines presented a low risk for consumption. This was noteworthy as "this is the FDA's first low-risk determination for enforcement discretion for an IGA in an animal for food use" [42]. It should be emphasised again that the FDA notes that this "is not a determination of 'safety' under the Federal Food, Drug, and Cosmetic Act, but is instead a determination that a product is low enough risk that it is not an FDA enforcement priority" [2].

The FDA structured its report in four sections: Molecular characterization, Phenotypic data and animal safety/health, Human food safety, and Environmental risk [1]. For the purposes of this article, only the first two sections will be considered. It is certainly worth noting in passing that the quick and unusual low-risk determination received by Acceligen™ was possible because the alterations made by the IGA not only already occur spontaneously but would be transferable, without resort to CRISPR-Cas9, through conventional breeding. Here, the FDA's accelerated decision-making differed from that in the case of AquAdvantage Salmon – one of the other two gene-edited animals to have been approved for food production by the FDA. However, in the case of AquAdvantage salmon, the promoter (i.e., a genetic component that "turns on the expression of a gene" [43]) came from another fish called the Ocean Pout (*Zoarces Americanus*) [43]. The 'PRLR-SLICK gene' case also differed from that of polled cattle, although even here the gene editing attempted to replicate a genotype identical to that achievable through conventional breeding [44]. In the polled cattle case, the difference was in the FDA assessments. The FDA detected in gene-edited dehorned cattle "two antibiotic resistance genes, along with various other gene sequences of bacterial origin. The inadvertently introduced bacterial sequences were found close to the editing site" [45].

The Molecular characterisation section of the FDA report sought to verify that PRLR-SLICK cattle had a similar genome sequence (although not identical) as that found in the conventionally raised cattle about which the FDA stated that "there is [a] history of safety" ([1], p. 3). In accordance with the latter statement, the FDA's verification covered the whole genome sequencing (WGS) data reported by Acceligen™ for the four calves that had been edited. The FDA makes four remarks in this section. First, as also reported by Acceligen™, among the four calves treated, only three were found to have been modified with the IGA. Second, as also reported by Acceligen™, the sudden death of one of the three edited calves was due to a heart defect that developed as a result of bovine congestive heart failure (BCHF). However, the FDA states that the genetic markers associated with BCHF were inherited from the calf's unedited parents, and consequently that the disease was unrelated to the IGA – according to the FDA risk assessment. Third, referring to the occurrence of unintended genetic alterations, the FDA records the same unintended alterations as those reported by Acceligen™. In addition to these, however, the FDA identifies "a few additional unintended alterations, including a duplication located in a repetitive intergenic region and indels (short insertions and deletions) in intergenic regions" ([1], p. 4). It then notes that none of these unintended alterations is expected to pose a health risk. The fourth, and last, consideration in this section of the report concerns the similarity with the naturally occurring SLICK genotypes. Having considered the biological variability issue, the FDA claims that "while the specific sequences of the IGA vary, the IGA contains premature stop codons predicted to result in PRLR protein truncation within the same amino acid range resulting from naturally occurring mutations" ([1], p. 4). For these reasons, it concludes that the molecular characterization analysis does not identify any safety concerns.

In the "Phenotypic data and animal safety/health" section of the FDA report, it is noted that calves carrying the IGA have a slicker coat as well as a higher heat tolerance than non-SLICK cattle do, with impacts observed on various factors, including ventilation rate, sweating rate, and body temperature. Animal safety considerations are based on data gathered from monitoring the animals' growth. The FDA notes that, excluding the calf that died of heart failure, no major health abnormalities are detected, and it is "concluded that risks to animal health for animals with the IGA are no greater than for conventionally raised cattle" ([1], p. 5).

The FDA report does not state which definition of "animal safety" it is employing. Nevertheless, the interpretation it works with is evident from various remarks that are made. Indeed, the analyses carried out only concern the observation of genotypic and phenotypic data, as well as the detection of unintended (or off-target) alterations. It emerges from the Risk Assessment that the conception of "animal safety" being applied is closely associated with a notion of health centring on the occurrence of unintended consequences of unintended alterations in the animals examined. The FDA report makes it possible to argue that no deadly health problems were detected in the two healthy, IGA-carrying calves.

One consideration is worth addressing at this point. As mentioned above, the evaluation conducted by the FDA was based on data reported by Acceligen™. The data related to four calves, of which, however, only two attempts at IGA successfully issued in cows that could be studied. Based on these observations, the conclusions drawn by the FDA could be regarded as corresponding to a tiny phase one trial of a new drug. The mention of drugs is not coincidental, but rather related to the fact that an intentional genomic alteration in animals is classified as an equivalent of introducing a new drug by the FDA and is hence regulated accordingly [46]. With such a small group of individuals being studied, the conclusions that can be drawn are limited. The FDA is aware of this. It recognizes "the limited nature of the dataset upon which this determination is based" ([1], p. 5). For example, on the basis of such limited data, only dramatic and very frequent changes can be detected. A small, but frequent, change would be easy to detect, while a rare, but big, but less frequent, change might be hard or impossible to identify. Moreover, distinguishing spontaneous mutations from off-target effects is complicated. Basically, the purpose of what appears to be the first phase of this trial for a new drug was to ensure that the IGA inserted via CRISPR-Cas9 does not lead to sudden death, or adverse effects, in the individuals "treated". For this reason, as previously mentioned, the FDA adds that if it "becomes aware of new information about risk, it may revisit these decisions" [2]. Further, although the occurrence and persistence of the SLICK variant in non-gene-edited animals (or, as the FDA puts it, "in nature") is a sign that cattle can live with it, this does not prove that it is completely safe. Once we have clarified this point, it becomes clear that the primary purpose of the risk assessment is to confirm that the IGA does not cause major and frequent detrimental effects.

If we take the FDA's evaluations to provide a first step, the discussion on how this gene editing may affect cattle welfare can then be further broadened. The next sections of this article will therefore deal with two concerns that can be raised within this wider welfare debate.

Concerns About Pleiotropy

One issue usually addressed within debates about genetic alterations is pleiotropy. *Pleiotropy* refers to the situation in which variation in a specific gene affects multiple phenotypic traits, even when these traits appear to be unrelated to each other [4, 5, 47]. A gene can have more than one function in an organism. Phenotypic variation may result from differences in how various forms of the genes perform these functions. Scholarly discussion of pleiotropy is wide-ranging. In this article we shall not engage in this discussion, however. Our aim is exclusively to provide a framework in which to present some considerations by drawing on the current literature. Therefore, we shall confine the discussion to issues relating specifically to the PRLR-SLICK cattle case.

It has been noted that there are several defining questions. One of these concerns what we mean when we say that a particular case of genetic polymorphism affects multiple phenotypic traits. "Many authors have distinguished between multiple independent effects of a mutation and multiple effects that depend on one another in a cascade. In the functional view of molecular gene pleiotropy, only the former is meaningful" ([5], pp. 67–68). In order to connect the case of the PRLR-SLICK gene to these remarks, and indeed to the broader debate on pleiotropy, we can refer to Jonathan Hodgkin's classification of the different types of pleiotropy [47]. Hodgkin proposes seven different modalities in which pleiotropy can occur, thus showing that there are several ways in which one gene can affect multiple phenotypic traits. The modality we are concerned with is what Hodgkin defines as "Combinatorial Pleiotropy" [47]. It is defined as follows:

> This term applies to the large number of cases where a single protein product interacts with a variety of different partners in different cell types, and as a result has altered specificity and/or biochemical activity in each different situation. Mutations affecting this protein will therefore have multiple and potentially diverse effects on a variety of tissues ([47], p. 503).

The problem then arises when the intentional genetic alteration, or IGA, of a gene not only involves a specific phenotypic trait – in this case hair growth – but also affects other traits. "Thus, if we switch the undesired gene variant out for the desired gene variant, we may produce an unintended phenotypic change along with our intended one" ([4], pp. 229–230).

As already mentioned, the IGA was carried out in the prolactin receptor gene (PRLR gene) and resulted in truncation of the prolactin receptor protein (PRLR protein). The hormone prolactin exercises its effects through interaction with the prolactin receptor. Prolactin has been documented to have pleiotropic effects [48, 49]. It has been reported that it performs more than 300 biological functions, and that these can be classified into six categories: water and electrolyte balance, growth and development, endocrinology and metabolism, brain and behaviour, reproduction, and immunoregulation and protection [48]. "Given the pleiotropic nature of prolactin, the mutation may affect other physiological characteristics. The liver is one organ that could potentially be affected because of the expression of PRLR" ([6], p. 1). Research conducted on 18 near-one-year-old heifers in 2020 aimed to address this possibility [6]. "Interpretation of the consequences of the SLICK1 mutation must include consideration of the fact that prolactin interacts with its receptor in multiple ways and the impact of the SLICK1-induced truncation of PRLR will depend on the pathways through which prolactin mediates specific biological effects" ([6], p. 6). Specifically, the study sought to discover what effects the SLICK mutation has on hepatic function. For example, prolactin acts on hepatic gene expression and influences growth and regeneration of the liver, sensitivity to insulin, triglyceride deposition, and carcinoma development. The authors argued that it is also important to determine whether the changes caused by the SLICK mutation are also likely to affect hepatic immune function. It should be noted, as the study points out, that "it is not possible to distinguish between effects of the SLICK1 mutation on the liver independent of its effects on body temperature [since] heat stress has been reported to alter the liver proteome in lactating cows" ([6], p. 6). The researchers concluded by that there is evidence, albeit modest, of an impact on liver gene expression. However, they added that further studies are required.

Although this is related more closely to production than cattle welfare, there is another concern about the role of prolactin to consider. One of the major functions of prolactin is to prepare the mammary gland for lactation during pregnancy [50]. Therefore, it is conceivable that the SLICK mutation will have a substantial effect on milk production in dairy cattle. Moreover, this effect could be easily overlooked in less selected breeds, where any direct effect on milk production may to some degree be offset by reduced heat stress.

In this section we have addressed the potential risk to cattle welfare posed by the pleiotropy of the gene affected by the IGA. Specifically, a recent study shows that prolactin is a gene whose variation can affect several biological functions, including those of the liver. Although the need for further studies was emphasised in the study, the researchers themselves noted the modest nature of the influence on liver function. Therefore, there is no evidence of a potentially fatal problem with cattle health, and the fact that animals naturally carrying SLICK variants thrive even without gene editing shows that cattle can survive with it.

The study presented above concerned the effects of the mutation on animals obtained through conventional breeding. It was therefore not referring to cattle edited with CRISPR-Cas9. The FDA points out that the SLICK variant obtained by Acceligen™ is not exactly the same as that observed in nature. The latter itself already occurs in various forms, so we can accurately refer to multiple naturally occurring variants. In any case, the "FDA concludes that the genotypic data demonstrate that the cattle with the IGA have a functionally equivalent genotype as conventionally raised cattle with naturally occurring slick mutations" ([1], p. 4). Moreover, as already mentioned, although the FDA has detected unintended alterations, none of these presented a major risk to animal safety. However, again the FDA's evaluation should be regarded as a (very limited) phase one trial, as it only involved two calves. Pleiotropy also remains an ongoing concern with regard to the implementation of CRISPR-Cas9 [4]. If the aim is to minimise risk (or even better, prevent it) in such a way that the IGA can be assumed to be an improvement in cattle welfare, not a worsening, the need for further studies is worth reiterating.

Improved Heat Tolerance and the Livestock Sector

In this section, potential risks to cattle welfare resulting from ways in which the livestock sector may use the IGA are discussed.

The result achieved by Acceligen™ may not be a one-off, and PRLR-SLICK cattle production based on gene editing and CRISPR-Cas9 may become widespread in the near future. The debate over gene editing is still heated, and there is no consensus within the scientific community on its merits. There are conflicting opinions on whether such editing can be considered a solution superior to that offered by conventional breeding. Indeed, there is no shortage of adverse views [51]. Some believe that gene editing may be an upgrade on conventional breeding [52–54]. *A fortiori*, they might add, with the advent of the CRISPR-Cas9 technique, which "appears to be an important step in the development of genome editing techniques [and] requires the least expensive reagents and is quick, precise and the easiest to implement" ([52], p. 83). In a 2019 article, which also refers to how genome editing could be employed to introduce useful alleles for heat tolerance, Alison Van Eenennaam argued:

> Modeling has revealed how the use of genome editing to introduce beneficial alleles into cattle breeds could maintain or even accelerate the rate of genetic gain accomplished by conventional breeding programs, and is a superior approach to the lengthy process of introgressing those same alleles from distant breeds ([54], p. 93).

The metaphor Van Eenennaam uses to describe the status of genome editing, as compared with other breeding techniques, is fascinating. "Genome editing can be envisioned as the cherry on top of the ice cream sundae of progress made using traditional breeding techniques and programs" ([54], p. 98), she says. She explains that at present, genome editing is primarily suited to dealing with qualitative traits that are controlled by a single gene (as is the case with the PRLR-SLICK gene). For this reason, "in the short term, therefore, it is likely that […] conventional selection will continue to make progress in selecting for all of the many small effect loci that impact the complex traits that contribute to the breeding objective" ([54], p. 98). With regard to the PRLR-SLICK cattle case, Peter Hansen has claimed that the advantage of gene editing over conventional breeding is that it makes it possible to "avoid the loss in production that results when breeds highly selected for milk yield, growth or carcass characteristics are bred to breeds highly adapted to hot environments that have not been intensely selected for production" ([55], p. 199).

Furthermore, in her article Van Eenennaam considers gene editing in relation to future growth in rates of consumption of animal products. An increase in global cattle-related food consumption could pave the way for massive interventions of gene editing in the breeding sector in the future. On one side, the growth of human population needs to be considered. In November 2022, global human population reached 8 billion. According to the latest projections by the United Nations, the world's population is expected to grow to 8.5 billion by 2030 and to 9.7 billion by 2050 [56]. Consumption of beef is projected to increase: according to OECD/FAO, global beef consumption is projected to reach 76 million tonnes by 2031 [57]. At the same time, consumption of dairy products (roughly 81% of which are cow's milk) is projected to reach 1076 million tonnes, increasing "faster than most other main agricultural commodities" [57].

Simultaneously, the rise in global population could lead to a decrease in arable land. As a result, some countries may convert land currently used for animal feed production to land used for growing crops for direct human consumption [58]. A second factor is climate change. The livestock sector is developing strategies to cope with the rise in temperatures and increased frequency and severity of heat waves. In this connection, a study of the dairy industry in Hawaii is worth mentioning [59]. The research was conducted by examining two milk farms located in two different areas on Hawaii Island (the largest of the Hawaiian Islands). With variables such as temperature, wind speed (WS) and solar radiation considered, "temperature-humidity index (THI) and WS variations in the hottest four months (June to September) were analysed to determine when critical thresholds that affect animal health are exceeded" ([59], p. 1). Considering the climatic differences between the two sites studied and noting a greater vulnerability to heat stress events in one than the other, the authors suggest that a possible adaptation strategy would be relocation of farms to areas with a more favourable climate.

How does this relate to the case we are currently analysing? PRLR-SLICK cattle, as a result of the IGA in the PRLR gene, have a shorter coat. Although further research is required "to confirm the effectiveness of the mutation for reducing negative consequences of heat stress" ([55], p. 199), SLICK variants that have occurred spontaneously have been shown to be associated with a higher heat tolerance. This higher tolerance would also seem to result in a reduction in milk yield under heat stress conditions [37, 41]. However, our point remains that if PRLR-SLICK cattle were less prone to heat stress, the need for relocation would become less urgent.

Research published in 2017 on the impact of the livestock sector on land use and climate change claimed:

> The increasing demand for livestock products has significantly changed the natural landscape.

> […] Land degradation is the deterioration of physical, chemical, and biological properties of soil. […] Land use change affects the natural carbon cycle, which consequently releases high amounts of carbon into the atmosphere, increasing GHG emissions ([60], p. 153).

In South America, for example, livestock ranching is one of the main factors in changes in land use [60, 61]. According to a 2013 FAO report, the intensity of emissions from cattle production in South America (100 kg CO2-eq/kg CW) is related to land-use change [62]. "Land-use change emissions are higher in this region due to deforestation caused by the expansion of grazing lands" ([62], p. 69). Having to deal with these issues, the farming sector may have to come up with a way out – a solution. If the projections [56, 57, 62] turn out to be correct, there would be three demands to consider: new space for growing crops for direct human consumption [58], increased demand for cattle products [57], and the need to reduce the impact of converting natural habitats.

However, farmers with cattle with higher heat tolerance could use this capacity to implement new strategies. This type of breeding intervention could be used, in other words, to avoid relocation to more favourable areas (or even for relocation to areas more exposed to excessive heat and currently not considered as a viable option). In this way, production could continue, while meeting the three above demands. However, while it is true that the SLICK variant promises to provide cows with a higher tolerance to heat, this certainly does not make the cows heat-immune. The improved phenotypic trait is useful insofar as it could provide relief in habitual areas, but it cannot be a solution to address stress in extremely heated zones. In this way, the risk is that PRLR-SLICK cattle would be exposed to a similar level of heat stress as that currently experienced by non-SLICK cattle. Hence, the expected welfare improvement would be non-existent.

Two considerations are worth addressing at this point. First of all, the presentation of the three factors has been useful in outlining possible premises of the case for moving these cattle to areas exposed to more heat. However, this does not mean that (1) the relocation will certainly occur. Nor does it mean that (2) the relocation could not also occur under the current or other conditions. Secondly, this risk should not be regarded as a direct consequence of the implementation of the SLICK variant, but as a secondary effect resulting from decisions taken by the livestock sector. The risk of stress for SLICK variant cattle will therefore depend solely on what changes are implemented in the production system.

Another possible welfare risk, and one that also relates to the way SLICK cattle are used in the production sector, concerns negligence. Since SLICK cattle cope better with heat, farmers could take advantage of them and economise on strategies usually adopted to keep herds cool [33]. How far, however, could such negligence extend before the cattle are harmed? The risks described in this section, as already mentioned, should not be seen as strictly, or directly, related to the development of the IGA. The intention is merely to emphasise that there may be certain threats to PRLR-SLICK cattle welfare should the limits of the IGA not be recognised, i.e., should it be overlooked that the genetic alteration offers only a slight improvement to the animal's ability to cope with heat stress, not complete safety. By considering potential actions of the breeders, it is possible to distinguish between a cattle gene editing project with the sole purpose of benefiting the sector's economy and one aimed at promoting animal welfare – albeit always within the existing food industry system. The positive primary effects (higher heat tolerance) would then be at risk of being nullified by negative secondary effects (similar or greater stress caused by animals being located in areas that are more exposed to excessive heat).

Conclusion

The Acceligen™ project involved only two calves. It could be argued that it is problematic to draw conclusions on the basis of such a limited group. However, this element of uncertainty is partly addressed by the FDA's Risk Assessment, which reports that some characteristics of calves with the IGA, such as the phenotypic traits, are similar to those detected in spontaneously occurring SLICK cattle [1]. Thus, although the research [37–41] referred to in this article on the benefits of the SLICK variant in increased heat tolerance was conducted on spontaneously occurring SLICK cattle, these conclusions should be translatable and apply to Acceligen™ cattle as well.

Studies on this issue should be carried out. The FDA Risk Assessment, particularly where any unintended alterations that have been detected are concerned, should at most be regarded as equivalent to a very limited phase one of a trial for a new drug. With only two individuals, only very common effects can be detected, so an element of uncertainty remains. Indeed, it is this element of uncertainty that has enabled some of the considerations about potential risks, such as the pleiotropy issue, to be discussed above.

As mentioned in the introduction, the aim of this article has been to examine the welfare consequences of the PRLR-SLICK cattle case. In this sense, there are two questions we should answer: an "empirical" one and a "philosophical-*cum*-ethical" one [10]. The first asks: Do PRLR-SLICK cattle have better welfare than non-SLICK cattle? The second asks: Having ascertained the welfare benefits, should the intervention then be pursued? However, there are two distinct answers here. The first is based on the information we currently have. According to what we know, does the IGA do what it is supposed to do – namely, improve heat tolerance to reduce heat stress and its adverse consequences? Considering the FDA Risk Assessment, we know that the IGA achieves the same enhanced heat tolerance as has been observed in cattle with the SLICK variant occurring spontaneously. Further, we know that although alterations have been detected, they are not expected to pose safety concerns. Given this information, how can we then answer the two questions?

Note here the "Principle for the Conservation of Welfare", first proposed by Bernard Rollin [63], and then revised by Adam Shriver [64]: "any animals that are genetically modified through the use of genetic technology, for purposes other than research, should be no worse off, in terms of suffering, than the parent stock was prior to genetic alterations" ([65], p. 40). According to our current knowledge, it would seem that this principle has been observed, and that – to adopt something Paul Thompson once wrote, in connection with the case of blind hens – as far as PRLR-SLICK cattle are concerned "the individual animals are better off than they otherwise might be" ([65], p. 311). The answer to the empirical question should, then, be in the affirmative. Therefore, we can claim that, since we have ascertained its welfare benefits, the IGA should be pursued: that is the answer to the philosophical-*cum*-ethical question.

The second possible answer does not rely on what we currently know, but rather depends on hypothetical outcomes. It has been shown that the spontaneously occurring SLICK variant affects liver function, although the research also demonstrated that the effects are modest [6]. However, since the FDA's findings are based on a study group of only two individuals, we can assume that the risks associated with the pleiotropic nature of prolactin [48, 49] are not entirely clear, and that pleiotropy may therefore still pose a welfare concern about cattle with the IGA. Therefore, and as has also been stated by the authors of the study regarding the effects of the SLICK variant on the liver [6], further research is required. Should any detrimental conditions resulting from the SLICK variant be identified, the positive effects of higher heat tolerance could be frustrated, or altogether outweighed by drawbacks. Furthermore, the improved performance of PRLR-SLICK cattle must not be conceived of as something that merely enable the livestock sector to use such higher heat tolerance without concern for animal welfare. Indeed, were the behaviour of livestock farmers to proceed in the direction of the examples described in the previous section (e.g. neglect of cooling measures and relocation strategies) there would be a risk that PRLR-SLICK cattle are exposed to levels of heat stress similar to those from which non-edited cattle currently suffer. Based on these assumptions about the potential risks, the second answer should be in the negative on both questions.

The considerations raised and examined by this article are not the only ones the PRLR-SLICK cattle case raises. As already noted in the introduction, the aim of the article has been to provide some initial insights with focus on welfare consequences in the actual, non-ideal world. Further studies and proposals, concerning genetics and welfare considerations, are warranted.

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Declarations

Consent for Publication Authors confirm that this is original work and not under submission anywhere else; they consent to the eventual publication of the paper in the journal.

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