Global Status of Gene Edited Food Animals and their Products

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Breeders have selected for desired changes to companion animal populations based on naturally-occurring variation.
Gene editing involves introducing a double-strand break in the DNA at a targeted location in the genome.

https://youtu.be/bM31E_LRszc
Gene editing allows the introduction of targeted double-stranded breaks in the genome.

- **Zinc Finger Nucleases**
- **TALENs**
- **CRISPR/Cas9**

**SDN-1**
- Nuclease-induced double-strand break

**“Knock-out”**
- Deletions
- Insertions
- Variable length indels

**“Knock-in”**
- Donor template
- HDR

**SDN-2,3**
- Precise insertion or modification

Introducing useful genetic variation into the germline of selected parents such that genetic improvement is inherited by the next generation is the ultimate goal of animal breeding.
Overview of literature review

• A search using Gene Editing in the Medical Subject Heading (Mesh), or gene edit*, or genome edit* or base edit* in the title or abstract and targeted to agricultural animals was performed in PubMed on July 21, 2023; and resulted in over 1,200 publications.

• After reviewing each publication, those that were exclusively for biomedical purposes, or where the edits were performed only in cells, or embryos that did not result in a live animals, or where edited animals did not survive beyond birth were excluded.

• The remaining 195 publications were categorized by editing system, species, purpose, type of edit (SDN-1,2,3), & country of first author.
NGTs used in the animal applications identified in the database (n=195)

- There were 59 applications (30%) where the editing was done in cell lines followed by cloning to produce an animal, all in mammals;
- 118 publications (61%) that edited developing embryos;
- 18 “other” approaches (9%) to editing, the majority of which were publications with avian species where editing was done in primordial germ cells.
- The majority ~ 75% of these applications were SDN-1 (147) aka knockouts; with 18 SDN-2, and 30 SDN-3 applications.
<table>
<thead>
<tr>
<th>Trait category</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abiotic stress tolerance</td>
<td>Resistance to abiotic stressors such as high or low temperature</td>
</tr>
<tr>
<td>Biotic stress tolerance</td>
<td>Resistance to biotic stressors such as bacteria, viruses and other pathogens</td>
</tr>
<tr>
<td>Color</td>
<td>Altered fur, hair, or skin color</td>
</tr>
<tr>
<td>Hypoallergenic</td>
<td>Reduced production or elimination of allergens in food products</td>
</tr>
<tr>
<td>Multiple</td>
<td>Applications that target more than a single trait category due to multiple target genes, or target genes with pleiotropic effects</td>
</tr>
<tr>
<td>Reproductive characteristics</td>
<td>Including changes in sexual characteristics such as sterility or the ratio of male to female offspring</td>
</tr>
<tr>
<td>Quality</td>
<td>Altered meat quality</td>
</tr>
<tr>
<td>Yield</td>
<td>Improved meat and fiber yield</td>
</tr>
<tr>
<td>Other traits</td>
<td>Traits not classified in the above categories, including welfare traits such as hornlessness and hypogonadotrophic hypogonadism as a pig castration free trait.</td>
</tr>
</tbody>
</table>
A recent literature review found 195 English-language category peer-reviewed publications producing gene edited food animals for agriculture – the purpose breakdown is below.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Common name</th>
<th>Species name</th>
<th>Number (N=195)</th>
<th>Yield</th>
<th>Reproduction</th>
<th>Biotic Stress/Abiotic Stress</th>
<th>Hypoallergenic/Quality</th>
<th>Multiple Traits</th>
<th>Other</th>
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<tbody>
<tr>
<td>Mammals (59%)</td>
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<tr>
<td>Pigs</td>
<td><em>Sus scrofa</em></td>
<td></td>
<td>52</td>
<td>16</td>
<td>4</td>
<td>18</td>
<td>9</td>
<td>3</td>
<td>2</td>
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<tr>
<td>Cattle</td>
<td><em>Bos taurus taurus</em></td>
<td></td>
<td>23</td>
<td>4</td>
<td>4</td>
<td>18</td>
<td>4</td>
<td>1</td>
<td></td>
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<tr>
<td>Sheep</td>
<td><em>Ovis aries</em></td>
<td></td>
<td>20</td>
<td>13</td>
<td>2</td>
<td>10</td>
<td>2</td>
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<tr>
<td>Goats</td>
<td><em>Capra hircus</em></td>
<td></td>
<td>17</td>
<td>11</td>
<td>2</td>
<td>8</td>
<td>1</td>
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<tr>
<td>Rabbits</td>
<td><em>Oryctolagus cuniculus</em></td>
<td></td>
<td>4</td>
<td>4</td>
<td></td>
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<tr>
<td>Avian (8%)</td>
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<tr>
<td>Chickens</td>
<td><em>Gallus gallus</em></td>
<td></td>
<td>13</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>4</td>
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<tr>
<td>Japanese Quail</td>
<td><em>Coturnix japonica</em></td>
<td></td>
<td>2</td>
<td>1</td>
<td></td>
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<tr>
<td>Duck</td>
<td><em>Anas platyrhynchos</em></td>
<td></td>
<td>1</td>
<td></td>
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<td></td>
<td>1</td>
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<tr>
<td>Aquatic Animals (29%)</td>
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<tr>
<td>Nile tilapia</td>
<td><em>Oreochromis niloticus</em></td>
<td></td>
<td>18</td>
<td>16</td>
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<tr>
<td>Atlantic salmon</td>
<td><em>Salmo salar</em></td>
<td></td>
<td>7</td>
<td>3</td>
<td></td>
<td>2</td>
<td>2</td>
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<tr>
<td>Common carp</td>
<td><em>Cyprinus carpio</em></td>
<td></td>
<td>4</td>
<td></td>
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<tr>
<td>Farmed carp</td>
<td><em>Labeo rohita</em></td>
<td></td>
<td>1</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>White crucian carp</td>
<td><em>Carassius auratus</em></td>
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<td>1</td>
<td></td>
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<tr>
<td>Mozambique Tilapia</td>
<td><em>Oreochromis mossambicus</em></td>
<td></td>
<td>1</td>
<td></td>
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<tr>
<td>Gibel carp</td>
<td><em>Carassius gibelio</em></td>
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<td>2</td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>Olive flounder</td>
<td><em>Paralichthys olivaceus</em></td>
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<td>2</td>
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<tr>
<td>Loach</td>
<td><em>Paramisgurnus dabryanus</em></td>
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<td>1</td>
<td></td>
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<tr>
<td>Channel catfish</td>
<td><em>Ictalurus punctatus</em></td>
<td></td>
<td>7</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
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<tr>
<td>Southern catfish</td>
<td><em>Silurus meridionali</em></td>
<td></td>
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<tr>
<td>Yellow catfish</td>
<td><em>Pelleobagrus fulvidraco</em></td>
<td></td>
<td>2</td>
<td>1</td>
<td>1</td>
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<tr>
<td>Sterlet</td>
<td><em>Acipenser ruthenus</em></td>
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<tr>
<td>Tiger pufferfish</td>
<td><em>Takifugu rubripes</em></td>
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<tr>
<td>Red sea bream</td>
<td><em>Pogrus major</em></td>
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<tr>
<td>Blunt snout sea bream</td>
<td><em>Megalobrama amblycephala</em></td>
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<tr>
<td>Rainbow Trout</td>
<td><em>Oncorhynchus mykiss</em></td>
<td></td>
<td>1</td>
<td></td>
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<tr>
<td>Redhead cichlid</td>
<td><em>Vieja melanura</em></td>
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<td>1</td>
<td></td>
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<tr>
<td>Royal farlowella</td>
<td><em>Sturisoma panamense</em></td>
<td></td>
<td>1</td>
<td></td>
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<tr>
<td>Oyster</td>
<td><em>Crassostrea gigas</em></td>
<td></td>
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<td></td>
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<tr>
<td>Insects (4%)</td>
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<tr>
<td>Silk worm</td>
<td><em>Bombyx mori</em></td>
<td></td>
<td>3</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Honeybee</td>
<td><em>Apis mellifera</em></td>
<td></td>
<td>4</td>
<td></td>
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<td></td>
<td>4</td>
</tr>
<tr>
<td>TOTAL</td>
<td></td>
<td></td>
<td>195</td>
<td>32%</td>
<td>20%</td>
<td>18%</td>
<td>12%</td>
<td>7%</td>
<td>11%</td>
</tr>
</tbody>
</table>
Country of first author on peer-reviewed publications producing Gene Edited Food Animals (and Their Agri/Food/Feed Products)
Global Regulatory Landscape for Products of Genome Editing

Countries with regulatory policy with exclusions
Countries with pending policies, regulations, or legal rulings
Countries with GMO only policy with no exclusions
Countries with regulatory policy with exclusions (plants only)

Slide courtesy Diane Wray-Cahen, USDA

Van Eenennaam USDA 2024
Global Regulatory Landscape for Products of Genome Editing

- **Canada**: not regulated unless product identified as novel
- **United States**: USDA - revised Rule for plants, some exclusions, ANPR for animals; EPA revised rule, some exclusions; FDA New Animal Drug Approval (animals)
- **Argentina, Brazil, Chile, Colombia, Paraguay, Guatemala, Honduras**: foreign DNA insertions generally regulated as GMO
- **Costa Rica, Ecuador, El Salvador, Uruguay**: similar under consideration
- **Norway**: proposed; tiered approach – notification, expedited, standard review; foreign DNA insertion regulated;
- **UK**: some exclusions for plants; animals under consideration
- **EU**: some proposed exclusions for plants; uncertain for animals
- **Israel**: foreign DNA insertions regulated; uncertain for animals
- **India**: draft policy; some exclusions
- **Japan**: foreign DNA insertions generally regulated as GMO
- **Korea**: draft policy; all LMO, some exclusions; Foreign DNA insertions generally regulated as GMO
- **Australia**: Code under review; templated changes currently regulated as GMO
- **Philippines**: foreign DNA insertions generally regulated as GMO; animal policies under consideration
- **South Africa**: current ruling for genome editing under GMO laws
- **New Zealand**: initial “non-GMO” ruling for some genome editing struck down by courts

Modified from Diane Wray-Cahen, USDA
Gene editing myostatin to obtain myostatin (Tilapia, Bream) and leptin receptor (Puffer) KO fish

Fish (Tilapia)
- Nile tilapia with increased fillet yield
  - Fish embryos injected with CRISPR/Cas9 mRNA
  - Deletions of nucleotides to knockout the gene
  - Increased growth rate and feed conversion
  - Product considered non-GMO in 2019

Puffer fish

Regional Fish

Red Sea Bream

Brazil

Argentina

https://sites.google.com/a/vt.edu/animalbiotechresources/2020-4th-intl-workshopVanEenennaamUSDA2024
Cattle with simple modifications were determined to be “non-GMO” in Brazil in 2021.

- Semen from a bull (Nelore) with double muscle
  - TALENs injection into the cytoplasm of IVF zygotes
  - Indels to knockout the myostatin gene
- Male and female with slick hair
  - CRISPR/Cas9 injection into the cytoplasm of IVF zygotes;
  - Mutations inserted in the prolactin receptor
- Both considered non-GMO in 2021

https://sites.google.com/a/vt.edu/animalbiotechresources/2020-4th-intl-workshop
Cattle with simple modifications were determined to be “non-GMO” in Argentina 2020

- **SLICK** edited Red Angus
- Double edited Celtic Pc polled/SLICK Holstein
  In partnership with Kheiron S.A.

Previous Consultation Instance: product under development
- Produced using TALENs
- 1) Celtic allele: hornless trait. Naturally present in Angus, Simmental, Limousin, Charolais and Galloway

June 2020 – no foreign DNA sequence and as such “no new combination of genetic material” And so considered “non-GMO”
FDA gives enforcement discretion to SLICK cattle submission by Acceligen (Recombinetics)

March 7, 2022

FDA Makes Low-Risk Determination for Marketing of Products from Genome-Edited Beef Cattle After Safety Review

Decision Regarding Slick-Haired Cattle is Agency’s First Enforcement Discretion Decision for an Intentional Genomic Alteration in an Animal for Food Use

March 7, 2022

Gene editing to produce Porcine Reproductive & Respiratory Syndrome (PRRS) virus resistant pigs

Technical considerations towards commercialization of respiratory and reproductive syndrome (PRRS) virus resistant pigs

Scaled production of pigs containing modified allele of CD163.

A. Donor → RNP → Surrogate → Piglets

- 4 porcine lines contribute zygotes
- Inject 1-cell zygotes with optimal dual-guide RNP complexes (No foreign DNA)
- Transplant 2-cell zygotes into surrogate gilts
- Mosaic piglets born 115 days later

B. Mosaic E0

- 2 unedited CD163 alleles
- 1, 2, >2 alleles edited (undesired and desired)
- 1 of 2 desired edited alleles
- 2 of 2 desired edited alleles

Scaled breeding steps for 1st, 2nd & 3rd generation of pigs to generate gene edited nucleus herd.

“Approximately 10–20 high genetic merit CD163°° boars across 2 maternal and 2 paternal lines are used to maintain a small nucleus population for multiplication and genetic improvement. Upon approval, these founders would be multiplied and distributed to producers for commercial production and sale using conventional breeding practices.”

Enforcement Discretion

GloFish (2003) Enforcement Discretion

Slide courtesy Diane Wray-Cahen, USDA

Genetically Engineered (rDNA)

- 2 Mosquitoes (2014, 2020, population control)
- Fall Armyworm (2021)
- Salmon (2021, somatotropin)
- Pig (2010, Environment, phytase)
- Salmon (2016, Food, somatotropin; 2013 Environment, somatotropin)
- Salmon (2015, somatotropin)
- Pig (2020, alpha-gal knockout)
- GloFish (2003) Enforcement Discretion
- 10 Silkworms (various, color, dye-retention)
- Silkworms (spider silk)
- Various species and traits in Phase 1, but none commercialized/deregulated

Genome Edited *

Brazil
- Tilapia (MSTN KO, 2019)
- Cattle (MSTN KO, 2021)
- SLICK Beef Cattle (2021)
- SLICK Dairy Cattle (2023)

Colombia
- PRRSv-resistant pig (2023)

USA
- SLICK Cattle (2022) Enforcement Discretion

Japan
- Sea Bream (MSTN KO, 2021; 2022 - variants)
- Tiger Pufferfish, Fugu (fast growth, 2021; 2022 - variants)
- Flounder (fast growth, 2023)

Argentina
- Tilapia (MSTN KO, 2018)
- SLICK Beef Cattle (2020)
- SLICK, Polled, Dairy Cattle (2020)
- Cattle (MSTN KO, 2021)
- Undisclosed, various species

Slide courtesy Diane Wray-Cahen, USDA
Summary

• Genome editing offers an approach to introduce useful genetic variation and alleles without the linkage drag typically associated with cross-breeding.

• Scaling useful edits to commercial livestock breeding programs will be technically complicated and expensive.

• Regulators in many countries consider simple edits (e.g. knockouts, moving allele from one breed to another) with no “foreign DNA” to be “non-GMO”

• The fate of genome editing in livestock will depend upon developing a risk-based regulatory framework that allows trade of animal products (meat, milk, eggs, and gametes)
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• Dr. Tad Sonstegard, Acceligen
• Dr. Bo Harstine, Select Sires Inc.

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Mitch Van Eenennaam Lab

USDA
United States Department of Agriculture

National Institute of Food and Agriculture