## Scientists may have found a radical solution for making your hamburger less bad for the planet

Researchers in California are working to genetically engineer the cow microbiome -- and in the process, eliminate methane emissions.

## By <u>Shannon Osaka</u>

## and Emily Wright

Photos and videos by Helynn Ospina for The Washington Post August 25, 2024.

DAVIS, Calif. — Sushi, a four-week-old Holstein calf, was lying in a pen under the hum of a metal fan when a group of professors and graduate students arrived to sample his stomach. The male calf greeted the researchers with a friendly nibble of their clothing, then flopped back down lazily on a bed of rice hulls.

But even as the calf slumbered, deep in his four-chambered stomach, minuscule organisms were hard at work. Fungi, bacteria, and other tiny creatures were breaking down feed into energy and chemicals, setting in motion an ancient process that today heats the Earth more than every flight across the world combined.

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Scientists here are hunting for a way to transform Sushi's gut — so that he no longer releases planet-warming methane.

The 125-pound calf belongs to the first wave of a multiyear, approximately \$30 million experiment by scientists at the University of California at Davis and the Innovative Genomics Institute to change the inner workings of the cow stomach. Cattle, one of the <u>most-consumed creatures</u> on the planet, produce enormous amounts of methane, a powerful greenhouse gas that is responsible for <u>30 percent</u> of global warming. Using tools that snip and transfer DNA, researchers plan to genetically engineer microbes in the cow stomach to eliminate those emissions. If they succeed, they could wipe out the world's <u>largest</u> human-made source of methane and help change the trajectory of planetary warming.



"It's completely out of the box," said Ermias Kebreab, a professor of animal science at UC-Davis. "Nobody has done it before."

There are approximately <u>1.5 billion cattle</u> on the planet. Their digestive systems are nothing short of miraculous — they can survive on grass, corn and alfalfa but also the battered byproducts of human crops: almond hulls, corn husks, even <u>sawdust</u>. The cows have help. A rich microbiome in the largest chamber of their stomach, known as the rumen, dismantles these foodstuffs and transforms them into usable energy.

"It's incredible," said Spencer Diamond, head of microbiome modeling at the genomics institute. "It works amazingly and never fails."

But the rumen has a dark side. The porous, fleshy chamber hosts single-celled organisms called archaea, which break down hydrogen and carbon dioxide, producing methane. Unable to process the gas, cows burp it up. The average cow produces around 220 pounds of methane per year, or around half the emissions of an average car; cows are currently responsible for around 4 percent of global warming, according to the Food and Agriculture Organization.

Dairy cows at the University of California at Davis. The university and the Innovative Genomics Institute are seeking to modify the inner workings of the cow stomach to reduce methane.

Partial solutions abound. Companies such as Impossible Foods and Beyond Meat have developed <u>plant-based beef products</u> that look, smell and taste like real meat.

Environmentalists have urged consumers to cut back on beef and opt for <u>lower-emissions</u> <u>chicken and fish</u> instead. But as countries grow richer, beef production continues to rise — in the past 15 years, by <u>13 percent</u> globally.

Adding seaweed, oregano, or garlic to cow diets can cut methane emissions, sometimes by up to <u>80 percent</u>. But only about 1 in 10 cattle in the United States — largely those producing milk — are fed every day by humans. The ratio is similar globally. The rest, mostly beef cattle, range free on pastures, surviving on grass and forage. Getting more than 1 billion free-ranging cattle to eat seaweed or garlic is logistically almost impossible. Diverse culture vessels are used to grow rumen microbes under anaerobic conditions at UC-Davis in June.

Enter a team of gene editors. Scientists envision a kind of probiotic pill, given to the cow at birth, that can transform its microbiome permanently. Using gene editing tools, researchers have already bred <u>cattle without horns</u>, or with special slick coats that help them stay cool amid rising temperatures. The current project doesn't target only a particular cow species — it takes aim at the microbiome itself, offering a solution that could apply to all of them.

Brad Ringeisen, executive director at the genomics institute, cut his teeth running biotechnology at the U.S. defense research agency DARPA, which helped pioneer transformative innovations including the internet, miniaturized GPS, stealth aircraft and the computer mouse.

"I'm taking the DARPA mentality here," he said. "Let's solve it for all cows, not just a fraction of the cows."

It starts deep in the cow's gut.

At the Davis dairy farm, two graduate students and a postdoc wrestled a long metal tube three feet down into Sushi's stomach and connected a pump. Out came a thin, oatmeal-colored liquid — a sample from Sushi's rumen, filled with microbes and partially digested feed.

The calf looked surprised, but not particularly uncomfortable. (This is one of the less invasive methods for testing the cow rumen; another involves making a port in the cow's side.)

Paulo de Méo Filho, a postdoc with a Brazilian accent, used a pipette as long as his arm to carefully move rumen samples into small vials. Then, wreathed in a faint string of fog, he dunked the vials into a bucket-sized container of liquid nitrogen to preserve them for DNA analysis.

For the past four weeks of his life, Sushi has been fed a few grams of oil distilled from red seaweed, one of the most tried and true methods to reduce the production of methane in cow stomachs.

Sushi's rumen fluid

Vials for genetic testing

Oil distilled from red seaweed

Sushi's feed

Now, the scientists are trying to understand exactly how that oil has transformed Sushi's gut. Then they plan to replicate those changes with gene editing.

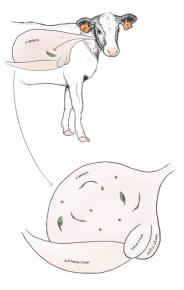
Despite how reliant humankind is on cows — they produce around 76 million tons of beef and 930 million tons of milk in a year — the inner workings of the cow's largest stomach chamber are still largely a mystery. Over millions of years, the cow microbiome has evolved to help the animal turn feed into energy. It is a chaotic soup of tiny organisms, all battling for limited resources, invisible to the naked eye.

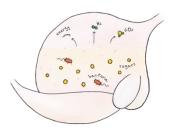
"The microbial world is a brutal, Mad Max wasteland," said Diamond, one of the genetic researchers. "Microbes just killing the crap out of each other."

It won't be easy to change. The system is complex, and scientists have rarely successfully transformed the microbiomes of ruminants like cows, sheep and goats.

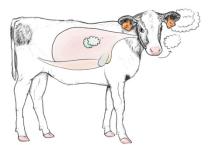
When cows eat, they chew their feed, mixing it with saliva, then swallow. (A cow can produce a whopping <u>40 gallons</u> of saliva per day, depending on its diet.) Some of that food is broken down further through "chewing the cud," or ruminating — in which the cow regurgitates some of its feed, gnashes it down further, then swallows it again.

Diagram showing a cow's stomach and the process of breaking down food into energy and methane.









All that partially masticated food ends up in the rumen.

Unlike the human stomach – which uses acid to break down food – in the cow rumen, the microbes do all the work. They ferment feed, much like a brewer makes beer.

Bacteria break down sugars into fatty acids, which provide energy for the cow, and two byproducts: hydrogen and carbon dioxide.

Archaea gobble up H2 and CO2 and combine them into methane, or CH4.

The cow burps methane out into the atmosphere, warming the planet and helping spur heat waves, flooding, and powerful storms.

But scientists point out that it doesn't have to be this way. There is nothing inherent to cattle that requires them to release planet-warming gases — it's just the buildup of hydrogen and the microbes that have evolved to consume that volatile gas.

"There's no reason a cow has to produce methane," Ringeisen said.

So what if scientists could just ... turn it off?

Mapping the microbiome

About an hour away from Sushi's pen, Diamond and his colleagues are using those frozen vials of half-digested food to map the inside of Sushi's stomach.

The Innovative Genomics Institute — co-founded by Jennifer Doudna, the Nobel-prizewinning chemist who helped pioneer CRISPR gene editing — occupies a tiered glass building in downtown Berkeley, Calif., just across the street from the main campus. Down in one of the laboratories, Brady Cress, the institute's head of microbiome editing, was playing an AI-generated country song about transforming the cow microbiome. The song's chorus came out in a tinny twang from his phone speakers: "Cow rumen, stop fumin'." CRISPR — clustered interspaced short palindromic repeats — is a set of DNA sequences found in bacteria and archaea. When combined with an enzyme known as Cas9, CRISPR can be used like a guided pair of scissors: slicing and dicing pieces of DNA before replacing them with new segments.

Nobel laureate Jennifer A. Doudna at Innovative Genomics Institute in Berkeley, Calif., in June.

Since Doudna and French scientist Emmanuelle Charpentier discovered CRISPR-Cas9 in 2012, the technology or its variants has been used to engineer glow-in-the-dark rabbits, caffeine-free coffee beans, and even the embryos of two twin girls to allegedly make them immune to HIV. (The Chinese researcher responsible for that last experiment was jailed for illegally using CRISPR in humans.)

Today, scientists at the institute are attempting to use gene editing tools to solve societal problems. Much has been made of how CRISPR could help solve human health problems, such as sickle cell disease, cancer or HIV. But some scientists here think the tool's most powerful application might be for methane. Methane, a potent greenhouse gas, stays in the atmosphere for seven to 12 years, while carbon dioxide can persist for hundreds of years. Curbing those emissions, which come from livestock, oil and gas, and land-use change, could also dramatically slow the rate of warming.

"I personally think this is the one that can make the biggest impact in the world," Ringeisen said. "Say you could wave a magic wand and eliminate all those emissions."

A gas chromatograph coupled to a mass spectrometer at UC-Davis is used to identify and quantify compounds that are produced by rumen microbes in June.

There are still many questions about the approach. "That's the holy grail, if it's possible to manipulate the microbiome of the rumen," said Alexander Hristov, a professor of dairy nutrition at Pennsylvania State University who isn't involved in the project. "But we have to keep in mind that this microbiome has developed over millions of years — it's very, very difficult to change or modify on a permanent basis," he said.

James Marsh, a professor of microbiome engineering at the Max Planck Institute for Biology in Germany, says that scientists are still in the early stages of applying genetic engineering to the entire microbiome. "We need to be able to apply it to all the organisms so we can really unleash the promise of microbial engineering," he said.

Down in the laboratory at the genomics institute, Diamond, dressed in a dark blue lab coat, cracked open a freezer about the size of an adult cow where genetic material is stored. After Sushi's rumen fluid is flash-frozen and processed in a lab at Davis, scientists transport the genetic material here and extract the DNA. Then, they begin to reassemble the species in the rumen from the ground up.

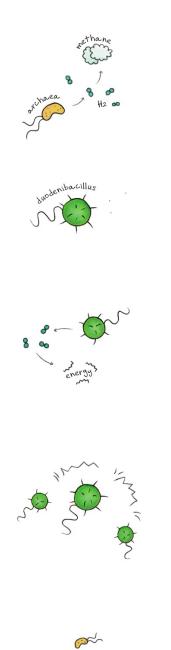
Researcher Spencer Diamond at Innovative Genomics Institute on June 28.

While scientists understand the basic contours of the cow rumen, they are still mapping all the species at work — a kind of microbial inventory of each bacteria, fungi, and archaea. That's essential both to understand which species are producing methane, and how to tweak the process.

"You're kind of on safari," Diamond said.

He describes it as assembling 100 jigsaw puzzles at the same time. The extraction process breaks each microorganism's DNA into hundreds of tiny pieces; with the help of a computer and machine learning, the researchers reassemble each of those genomes. From there, they can see how the microbiome has changed under the seaweed treatment — and how to mimic it.

Diagram showing the duodenibacillus bacteria and how it produces less methane in the digestion process.



So far, they found that seaweed oil blocks an enzyme that archaea use to process hydrogen. Without it, the number of archaea plummet.

That leaves more hydrogen for bacteria, like this one called the *duodenibacillus*. Like archaea, the *duodenibacillus* gobbles up hydrogen – but instead of turning H2 into methane, it turns it into more energy for the cow.

Scientists believe they can mimic this effect with gene editing. They could insert edits into *duodenibacillus* – or a similar organism – that make it faster and stronger, helping it to outcompete archaea and eat even more.

Without H2, the archaea will have nothing to eat – and nothing to turn into methane. Story continues below advertisement Engineering a competitor

When removing an unwanted species from an ecosystem, Diamond says, the microbial world is much like the macro world: You can target the species in different ways.

A community with an influx of deer could start a hunting program — in the microbial world, that's the equivalent of inserting a gene into the methane producers that wipe them out. But just as it's hard to find and target all the deer, it's difficult to ensure that a gene editing tool reaches all archaea. "It's actually very difficult to edit every single microbe of a group," Diamond said.

An alternative — and one that Cress and Diamond think is more likely to be successful — is to introduce a competitor, a kind of microbial elk that could devour the hydrogen before the archaea get a chance to turn it into methane. A bacteria like the *duodenibacillus* could be gifted gene-edited advantages that boost it above the archaea.

Ermias Kebreab, a professor of animal science at UC-Davis. where researchers want to genetically engineer microbes in the cow stomach to eliminate methane emissions. "It's completely out of the box," he said.

"We want to trigger a permanent change," said Matthias Hess, a professor of animal science at UC-Davis who will test some of the treatments in his lab.

The ideal treatment would be a kind of early-life probiotic treatment for a cow — a capsule that a calf could swallow that would create a lifelong change in its microbiome. While only a small number of cattle are fed every day, the majority of them receive at least one vaccine in early life. The researchers envision a probiotic pill administered with those early vaccines, reaching cows that otherwise will spend their entire lives on pasture.



Calves rest in

their pens in June as part of a collaborative project between UC-Davis and the Innovative Genomics Institute.

Such a probiotic could also improve a farm's productivity. Cattle can lose up to 12 percent of their energy through burping up methane; other ruminants, like sheep and goats, also lose energy in this way. "If there is a way to redirect that hydrogen and convert it into milk, meat, wool — it would be much more accepted by farmers," Kebreab said.

Early treatments will be tested on the cattle at Davis, with researchers tracking their burps to evaluate the drop-off in methane emissions.

There is still a long way to go. While scientists have proved that they can gene-edit microbes, researchers have so far only shown that they can edit a small fraction of the microbes in the cow gut — or the human gut, for that matter. Institute researchers are developing microbial gene-editing tools, even as they are mapping the species of the microbiome. They are building the plane while flying it.

The teams have received <u>enough funding</u> for seven years of research. The project started last year, and they hope to have a trial treatment ready for testing in cows in the next two years.

Matthias Hess, a professor of animal science at UC-Davis on June 25. Hess will test some of the treatments in his lab.

For some skeptics of gene editing, a kind of gene-edited, supercharged probiotic pill might seem more palatable than modifying an entire species of cow, or growing meat in a

bioreactor. But fears of unintended consequences remain, even for the researchers. "We have to be mindful of the power of these technologies," Diamond said. "People will fear the unknown."

Still, the promise of editing microbes is tempting. It's not just cattle that produce methane, but also goats, sheep, ancient outposts of permafrost in the Arctic, and boggy wetlands in temperate regions. The lessons from cows, Ringeisen says, could help design interventions for other animals and even ecosystems. "I'm convinced this is a solvable problem," he said. Back at the Davis dairy farm, his rumen fluid safely frozen in labs around the San Francisco Bay Area, Sushi the calf folded his legs under him and laid down on his bed of rice hulls for a nap. A fly landed on his ear and he twitched it off. All around him, the cows at the dairy were breathing out methane.



Sushi rests in his pen.

## About this story

Data on the impact of cow and ruminant methane on global greenhouse gas emissions is from recent Food and Agriculture Organization reports. Our illustrations of the processes in cow digestion were based on conversations with Spencer Diamond and Matthias Hess. All illustrations are simplified representations of the cow rumen digestion.