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Agriculture and Natural Resources

California Dairy Newsletter

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What is Gene Editing?

Alison Van Eenennaam, UCCE Animal Genomics and Biotechnology Specialist

A new set of technologies that collectively are being used to edit genes are deriving a lot of media attention. As the name "gene editing" suggests, these technologies enable researchers to add, delete, or replace letters in the genetic code. In the same way that spell check identifies and corrects single letter errors in a word or grammar errors in a sentence, gene editing can be used to identify and change the letters that make up the genetic code (i.e. DNA) within a species.

Gene editing has many potential applications. For example, it can be used to correct diseases and disorders that have a genetic basis. It could also be used to change one less desirable allele of a gene to a more desirable allele without the need to introgress (repeatedly backcross) or bring in that allele through outcrossing with an animal that carries the desirable allele.

Gene editing is different from traditional genetic engineering. Continuing with the analogy of a word processor, genetic engineering enables a gene sequence of "foreign DNA" to be "cut and pasted" from one species to another, whereas gene editing can add, delete, or replace a series of letters in the genetic code. There are many potential uses of this technology ranging from human medicine to plant and animal breeding.

The basic idea behind gene editing is that molecular scissors called nucleases are used to cut DNA at a specific location in the genome based on recognition of the specific, unique target DNA sequence. The cut site is then repaired using the DNA repair mechanisms of the cell. These repairs can be directed to introduce, delete, or replace a series of letters in the genetic code. This essentially enables the introduction of known, desired alleles based on what is understood about naturally-occurring genetic variation in the target species.

<u>Where might gene editing be used in animal breeding?</u>

The currently available set of gene editing technologies (zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and clustered regulatory interspersed short palindromic repeats (CRISPRs) associated system have been used for a relatively small number of livestock applications to date.
Gene editing has been used to produce genetically hornless Holstein dairy cattle by introducing the naturally-occurring Angus "polled" allele into the gene that is responsible for horns and to generate pigs with a single base deletion in a gene that may confer resilience to African Swine Fever Virus. It has also been used to introduce changes in the *myostatin* gene in sheep and cattle. As the Latin origin of the word *myostatin*

(muscle/stop) suggests, turning off this gene results in muscle growth. Naturally-occurring mutations in this gene have historically been selected by conventional animal breeders and are the genetic basis for the "double muscled" phenotype that is seen in cattle breeds like the Belgian Blue, and the "bully" phenotype in whippet dogs.

In this way, gene editing really mimics the natural processes that form the basis of selective breeding programs, and for that matter, evolution itself. Breeders work with the genetic variation that exists within a species, and that genetic variation ultimately arises from naturally-occurring mutations. Although the word "mutation" sounds negative, it simply refers to variations in DNA sequences. These variations, or mutations, are responsible for virtually all genetic differences that exist between individuals, such as having blue eyes instead of brown.

Although different mammals have many of the same genes, many people do not appreciate that the genetic code that makes up those genes differs among animals of different breeds, and even among animals within the same breed. In fact, with the exception of identical twins, there are literally thousands of DNA sequence variations between two individuals of any species. For example, an enormous number of genetic variants have accumulated within cattle since the advent of domestication and selective breeding due to the naturally-occurring processes that lead to a small number of mutations each generation. In one recent analysis of whole-genome sequence data from 234 taurine cattle representing 3 breeds, more than 28 million variants were observed, including insertions, deletions and single nucleotide variants. A small fraction of these mutations are those that have been selected by breeders; most of them are silent and have no impact on traits of importance to breeding programs. Occasionally, such mutations result in a genetic condition such as red or black coat color or an undesirable disease condition such as dwarfism.

How might gene editing intersect with conventional breeding?

Data coming out of some of the large-scale genomics and sequencing projects are revealing situations in which the sequence of one naturally-occurring allele results in a superior phenotype to that observed when animals inherit an alternative allele of that gene. It is envisioned that it might be possible to edit an animal's genome to the superior allele, and to do that at several genomic locations, or for several different genes. The advantage of gene editing over conventional selection to move these naturally-occurring alleles from one animal to another is that favorable alleles rarely all occur in one single individual and editing offers the opportunity to increase the frequency of desirable alleles in an individual or a breed more rapidly than could occur through conventional breeding.

One could potentially envision editing several alleles for different traits – disease resistance, polled and to correct a known genetic defect – all while using conventional selection methods to keep making genetic progress towards the selection objective. One study found that combining gene editing with genomic selection could improve the response to selection four fold after 20 generations.

Gene editing offers an approach to translate the thousands of SNP markers discovered through livestock sequencing projects, the information obtained from numerous genome wide association studies, and the discovery of causative SNPs (QTNs) into useful genetic variation for use in animal breeding programs.

Will gene editing be regulated?

At the current time it is unclear whether gene editing will be formally regulated. Animal breeding per se is not regulated by the federal government, although it is illegal to sell an unsafe food product regardless of the breeding method that was used to produce it. Gene editing does not necessarily introduce any foreign genetic DNA or "transgenic sequences" into the genome, and many of the changes produced would not be distinguishable from naturally-occurring mutations. As such, it does not fit the classical definition of genetic

engineering. It is not evident what unique risks might be associated with an animal that is carrying a naturally-occurring allele or a gene deletion produced through gene editing. Governments and regulators globally are currently contemplating whether gene edited animals should be regulated, and if so how and by whom. This question is of course important from the point of view of both technology development and innovation, and international trade.

Silage Pile Underlay Plastic: Is an Enhanced Oxygen Barrier (EOB) of Value?

Peter Robinson, UCCE Dairy Nutrition Specialist & Nadia Swanepoel, Dept. of Animal Science

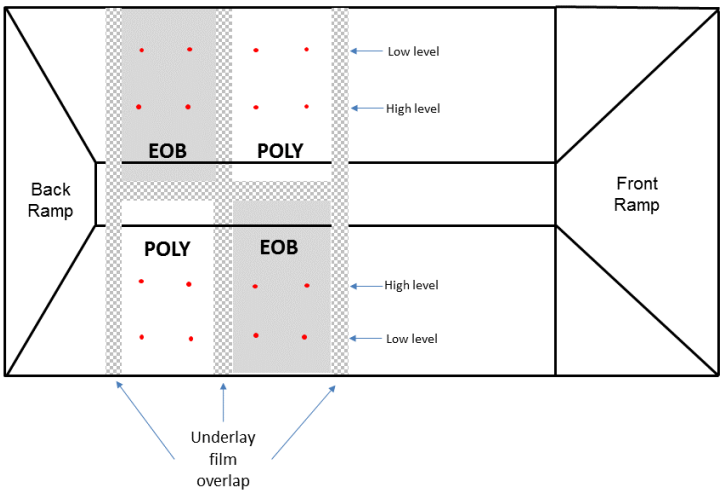
Corn silage is a very important ensiled crop in California. However spoilage is an economic loss to dairy farmers. One of the critical points to control spoilage in silage is to limit oxygen entry since it supports growth of aerobic microorganisms and the resulting heat production can lead to silage with degraded nutritional quality, as well as higher shrink losses. A relatively simple practice to reduce spoilage which has gained wide use on California corn silage piles is use of a thin inner plastic film with enhanced oxygen barrier (EOB) properties between the silage and the main plastic cover.

While there are many (21) published studies which claim to have compared thin plastic silage films with and without EOB characteristics, only one actually compared this effect without other simultaneous changes, and it was in 20 pound bag silos!

Surface spoilage not impacted by underlay film prior to exposure

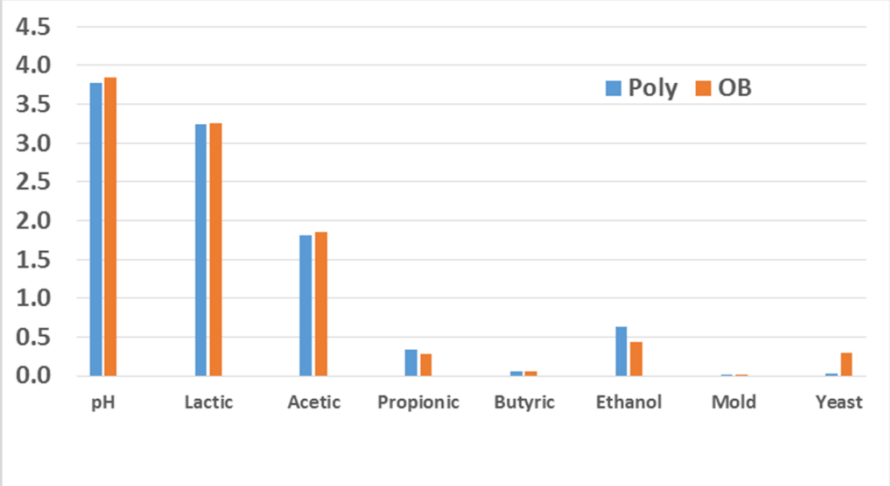
We wanted to measure fermentation characteristics of corn silage which are indicative of silage deterioration as impacted by use of thin plastic underlay films with or without EOB characteristics. To do this, 4 large wedge type corn silage piles were constructed in the Northern San Joaquin Valley to examine surface spoilage of silage through ~6 months post pile building as impacted by the plastic underlay film. The piles, 5,650 tons on average, were covered within 48 hours near one end with alternate coverage (Figure 1) of a clear, pliable polyethylene film (POLY) of 40.6 microns ('HiTec') or an EOB plastic film of 45.7 microns ('Silostop'). Sections on the pile surfaces were created with ~50 foot wide plastic sheets of underlay film with ~3 foot overlaps at each side and at the top of the piles. All piles were covered with 127 micron white/black plastic, white side out, and covered with side-by-side rings of ½ tires – with treatment overlaps covered with 2 rings of ½ tires.

Figure 1. Coverage plan on 4 silage piles (dots are the approximate core points).



About 2.8 and 5.9 months after covering, the pile surfaces were core sampled in each of the four sections of each pile by coring through the silage cover plastic four times at two levels, being $^{1}/_{3}$ of the way up each side (Low level; 2 cores/section) and $^{2}/_{3}$ of the way up the side (High level; 2 cores/section). These are the red dots in Figure 1. Each coring consisted of first coring to a depth of 10 inches (outer core), followed by re-coring of the same hole to 20 inches (inner core).

Figure 2. Impacts of a POLY versus EOB underlay film on measures of silage deterioration (lactic, acetic, propionic, butyric, ethanol as % fresh weight; mold and yeast as CFU/g fresh weight). [There were no statistically significant differences between films.]



Overall (Figure 2) there were no differences in any measured characteristics of the silage under the POLY *versus* EOB films.

Surface and peripheral face spoilage is not impacted by underlay film at exposure.

Not deterred by this result, a ~7,800 ton pile of corn silage was constructed to examine surface, face edge and deep mass spoilage of silage during pile feedout as impacted by underlay film. Near one end of the pile it was alternately covered with Hi-Tec or Silostop films within 48 h to create one section of each treatment on each side of the pile. The balance of the covering process was as previously described.

Approximately 0.4 and 0.6 of the distance into each of Sections 1 and 2 at feedout, the exposed face and surface behind the face (i.e., 1.5 feet into the plastic cover, or ~5 feet from the exposed face, since ~3.5 feet of silage had been exposed by surface plastic removal, were core sampled according to a grid to create silage samples which represented the deep mass, outer edge of the face, and the surface behind the exposed face for each side of each section. Each of the 4 coring events (i.e., 2 per experimental section) used the same coring device described earlier.

Underlay film had no impact on any response parameter, except that the aNDF level of the silage under the POLY film was slightly higher than that of the silage under the EOB film.

What have we learned?

The POLY and EOB silage underlay films had similar impacts on measures of silage deterioration of corn silage pile surfaces prior to opening, or during pile feedout, as well as ~25 inches under the pile surface at the exposed face or in the deep silage mass of the pile. Both underlay films were associated with well-preserved silage with little sign of deterioration.

However, because the surface 20 inches in direct proximity to the exposed face had deteriorated regardless of underlay film, further investigation was undertaken to determine why it was occurring. The results of this investigation will be discussed in the April issue.

Winter: The Perfect Time to Review Your Operation and Maintenance Plan

Deanne Meyer, UCCE Livestock Waste Management Specialist

It's that time of year where people resolve to improve. Did you make a resolution to improve the management of your dairy? Perhaps this is a repeat resolution from years gone by, when you challenge yourself to always do better than you did the year before. Or perhaps this is just the year to be sure something beneficial is accomplished in the year ahead. Regardless of your motives, it's important to review and update your Dairy's Operation and Maintenance Plan (O & M).

Most dairies have and O & M Plan. It's been a few winters since we had sufficient rain to challenge infrastructure management at most dairies. Methodically review each area of the dairy. Is your O & M sufficient in the milking parlor, animal housing areas, feed and manure storage areas, and land used for application of manure? Is maintenance conducted regularly to ensure the Standard Operating Procedures for facility management are met?

Liquid storage structures are very important on dairies and especially important when it's rainy. Remember to visually review liquid storage structures weekly during the rainy season and document status of your lagoon conditions (required in the Central Valley and for dairies in the San Francisco Bay Region). Ponds should have markers in them to identify if sufficient freeboard is maintained. The marker requirements are described in each General Order for Waste Discharge Requirements or Conditional Waiver. *Visual observation of markers lets you know if you do or don't have sufficient freeboard for structural integrity and to hold a 25 year, 24-hr storm event.* Most O & M have language about minimizing vectors (mosquitoes, burrowing animals, flies, etc.). *Flies can be a nuisance for operators and animals. Mosquitoes may spread disease. Burrowing animals cause or use holes in the sides of ponds. These holes interfere with the storage capacity of ponds and can definitely impair the structural integrity of ponds. Little holes in ponds may be eroded away and result in breaching or loss of side walls in both above ground ponds and in-ground ponds.* In spring or summer, when ponds are cleaned out, be sure liner integrity is maintained. Contractors hired to empty ponds, install equipment, or to remove sludge should always be reminded of the importance of maintaining pond liner integrity. *Maintaining pond integrity is important to keep it structurally sound as well as prevent leaching of nutrients to groundwater.* You'll also want to take stock in your spare parts (belts, bearings, switches, plumbing, fuses, etc.) inventory that is maintained on-site and identify which potential parts are available as needed from suppliers. Generators and pumps may well be used a lot this storm season.

When storage capacity is getting short, it's important to review the facility Waste and Nutrient Management Plans to identify if parlor water conservation will yield storage benefits or if diversion of roof water would be beneficial. Also, remember to sample liquid manure to identify nutrient concentrations (especially if you'll be land applying the material). The extra rain this winter likely altered your lagoon water nutrient concentration.

Lastly, don't forget to use your life lines. Have discussions with your environmental consultant if you're concerned about facility management or want to make improvements. Immediately contact your environmental consultant or trade association field staff as well as the Regional Water Quality Control Board if you have an off-site discharge, as compliance samples and documentation are needed under these circumstances.

Here's hoping your O & M is working well for you and that we have a heavy snow pack with a slow water release in spring!

What Do You Do if You Dump Your Pond?

Deanne Meyer, UCCE Livestock Waste Management Specialist

Since May 2007 there's been much written about the Dairy General Order in the Central Valley. Producers are curious about what happens when people have more liquid than their pond will hold. The old cliché of the best defense is a good offense applies. First and foremost, manage pond storage capacity so you have enough room. Implementation of your Operation and Maintenance Plan (discussed elsewhere in this newsletter) is essential during rainy weather. If storage is a short commodity, do your best to minimize daily fresh water entering the pond and maximize diversion of roof run-off where and when feasible.

What happens if you apply manure beyond the amounts identified in your Nutrient Management Plan? Notify the Regional Board immediately! Based on Violation Notices written by the Central Valley Regional Water Quality Control Board "Pursuant to Monitoring and Reporting Program (MRP) Section C, 'Priority Reporting of Significant Events,'" the written report required by this section is due within two weeks after the date you became aware of the noncompliance incident. You must provide this report **immediately** to avoid accumulating additional potential liability for violations of the General Order. The report should contain a description of the noncompliance, its causes, duration, and the actual or anticipated time for achieving compliance, and details of the steps that you have taken or intend to take, in order to remediate the problem (please see items 1 to 8, page MRP-11 of the Order). **Remediation must include removal of all excess manure from the cropland.** In addition, the "quantity of manure discharged and total amount of nitrogen within the manure must be included in the report." Note--the remediation of removal of excess manure applied requires scraping manure and soil from the field and exporting it off-site or to a location on-site where the nutrients may be used. This relocation activity also requires a paper trail to document what was done. The Violation Notices further identify the need for detailed soil sampling requirements and that the operator will continue to accrue potential daily liability until they comply with the Notice of Violation. A facility is subject to additional enforcement action, and/or termination of authorization to discharge according to the General Order, Provision E.10 if there is no compliance with the Violation Notice.

Nutrient Management Plans are designed to help you manage your nutrients and comply with the General Order. If your facility has challenges holding liquids until it's appropriate to land apply nutrients, it'll be important to have a conversation with your design engineer and identify what options exist for improving holding capacity for your operation.

Recruitment Underway for Two New Dairy Advisors in the San Joaquin Valley

The University of California, Division of Agriculture and Natural Resources is seeking UC Cooperative Extension (CE) academic advisors to conduct multi-county-based extension and applied research programs focused on Dairy Science. These positions will provide programs across a spectrum of industry issues as they relate to dairy production systems. Successful programs will address production issues and sustainability in an integrated approach that will consider economic viability and conservation of natural resources including land use, air, water, and energy.

A minimum of a Master's Degree is required, though other advanced degrees are encouraged, in disciplines such as Dairy or Animal Science, or a closely related field. Excellent written, oral and interpersonal communication skills are required. Demonstrated ability in applied animal science research and extension experience are desirable.

Positions will be located in **Tulare** (Tulare/Kings/Kern Counties) and **Fresno** (Fresno/Madera Counties).
For more information, or to apply, please visit: http://ucanr.edu/Jobs/Jobs_990/

2016 Golden State Dairy Management Conference

March 8-10, 2016

Embassy Suites Monterey Bay - Seaside

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$220 before Feb. 22
$245 after Feb. 22
$270 for on-site registration

Reserve Your Room soon!

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- *Kayaking and whale watching...*
- *Exploring Cannery Row...*
- *And much more!*

California Topics for California Dairies

Conference Highlights

Visit our website, http://ucanr.edu/sites/CAdairyconference/ to register. You will also find up to date information on the agenda, continuing education credits, and the lodging block.

Who should attend: Dairy producers, nutritionists, veterinarians, and other members of allied industry who are interested in topics related to dairy production in California.

Lodging

Embassy Suites Monterey Bay - Seaside
1441 Canyon Del Rey Blvd, Seaside, CA 93955

Call the Embassy Suites at 1-831-393-1115 and use our Group Code: **CD1**.

Continuing Education

ARPAS and CCA credits have been requested.

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California Dairy Newsletter

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