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Technological advances in bacterial identification

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Milk laboratories across the country have moved away from standard culture methods using agar plates and biochemical tests for bacterial identification to more technology driven methods like MALDI-TOF MS (Matrix Assisted Laser Desorption/Ionization-Time of Flight Mass Spectrometry). There are many reasons for this shift including reduced human resources, far more rapid and robust results, as well as a substantial increase in accuracy. However, the downside is difficulty in interpretation of the results. The purpose of this article is to take a closer look at the MALDI-TOF MS method used for bacterial identification and discuss some guidelines for interpretation of results.

Traditional methods of identifying bacteria are incredibly time consuming and in the cases of some bacteria, can take upwards of one week for results to be finalized. The use of a system like MALDI-TOF MS substantially reduces the wait time and increases the specificity of identification. The culture method begins the same with traditional agar plates, but then instead of running a host of biochemical and microscopic tests, the colony of bacteria are exposed to the MALDI-TOF MS procedure. The technique is designed to ionize the particles of the bacterial colony and

subsequently separate the particles according to their mass-to-charge ratio. This is measured by the amount of time it takes for the ions to travel to a detector at the end of a tube. The resulting spectrum of mass-to-charge ratios and light intensities are compared to a huge database of known organisms. Successful identification of the bacteria relies heavily on the comparative database. The more samples and organisms in the database, the better the output will be.

In traditional culture methods, results would often end without a species identification as in with the coagulase negative staphylococci (CNS). Historically, CNS have been considered what milk bacteriologists call “minor pathogens”. As the nomenclature suggests, these bacteria are thought to have a lesser effect on the udder and milk quality than the opposing group called “major pathogens”. Major pathogens include the coagulase-positive *Staphylococcus aureus*, as well as bacteria like *Escherichia coli*, and *Streptococcus uberis*. However, research over the last decade+, have shown us that not all CNS are equal. There are certainly some CNS that behave more like major pathogens. Therefore, proper species identification of CNS is critical for optimal milk quality monitoring.

Because the comparison database consists of over 2500 microbial species, MALDI-TOF MS results can be very difficult to pronounce much less interpret. In many situations, the results may list bacteria that seem uncommon or novel. However, these bacteria only appear that way because we did not have the microbiological methods available to properly identify these pathogens

previously. If your diagnostic laboratory uses this platform for bacterial identification, I recommend you work closely with your herd veterinarian or milk quality consultant to help you discern the results. They can help you to find groups of like-bacteria to provide knowledge with regards to prevention, control and treatment strategies.

The use of MALDI-TOF MS is growing among milk diagnostic laboratories and provides consistent, accurate, and rapid results. Species-level identification of bacteria will continue to increase as more information is added to the referent database. This means the sky is the limit with the ability of this system to properly identify mastitis-causing pathogens. So, although the results may seem intimidating when you try to pronounce the bacterial pathogens, I encourage you to let your herd health and milk quality teams assist in analysis interpretation.

Ruminal fiber passage rate: A double-edged sword for cattle methane emissions?

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To moderate climate change, over 100 countries pledged to reduce methane emissions by 30% by 2030 at the 26th annual UN Climate Change Conference of the Parties in 2021. Enteric methane, or methane naturally produced during ruminal fermentation, is the largest source of methane among dairy sectors, and makes up 2.5% of total U.S. greenhouse gas emissions. With regulations on greenhouse gas emissions on livestock operations inevitable, animal researchers must determine sustainable methods to reduce dairy cattle enteric methane emissions easily implementable on farms today. Research areas for Virginia Cooperative Extension

mitigating these emissions include selective breeding, which can take generations to have an effect, or dietary alterations like feeding costly additives that directly impair methane-producing bacteria vital for maintaining the rumen environment. A better and more sustainable strategy to decrease enteric methane production is to increase the ruminal fiber passage rate of dairy cattle rations. Despite the accompanied pros, as will be discussed, there are some potential pitfalls to increasing ruminal fiber passage rate. This article aims to outline the manipulation of ruminal fiber passage rate to reduce enteric methane emissions and its secondary influences on animal production.

Ruminal fiber passage rate is the speed at which most structural components of a feed (cellulose, hemicellulose, and lignin; **NDF**) escape the rumen. Passage rate is heavily modulated by the dietary forage quality and NDF concentration such that better quality forages that contain less and/or more digestible NDF have faster passage rates. This is due to improved chewing and rumination. The microbiome is also able to break down the fiber components and reduce the bulk in the rumen more efficiently. This allows the resulting smaller particle sizes from microbial degradation and rumination to escape to the remaining digestive tract at a faster rate. Selection of forages based on their NDF concentration and digestibility, and managing forage harvesting time to alter these properties, are strategies to alter ruminal fiber passage rate. Decreasing dietary forage particle size by chopping forages smaller or decreasing forage concentrations in favor of less fibrous carbohydrates are also strategies to increase the passage rate.

Several animal feeding studies evaluating alternative forages' effects on enteric methane production state that reduced enteric methane production is due to an increased ruminal fiber passage rate. Despite there being a lack of hard data for this statement within these studies, lab- and model-based results provide a foundation for

this. A lab-based study used culture fermenters to simulate rumen fermentation under low (2.5 %/h) and high (5.0 %/h) passage rate conditions with the same feed source. The results showed that the faster passage rate resulted in a 33% decrease in methane production while NDF digestibility remained the same. A modeling approach similarly determined that methane emissions decrease with a faster passage rate (2.5 vs. 3.4 %/h). Accounting for several other conditions within the rumen environment, it was estimated that the increased passage rate decreased enteric methane production by 17%. With approximately 9.4 million dairy cows in the US, increasing ruminal fiber passage rate of dairy rations by just 1 %/h could potentially reduce annual methane emissions by 366,000 tons or more. That's approximately 12% of total cattle enteric methane emissions in the US!

Despite the benefits of reducing enteric methane production by increasing passage rate, other factors regarding animal production need to be considered. An increased passage rate is associated with increased feed intake due to the emptying of the rumen. A great outcome of this is increased milk yield. However, research has found that when forages with faster passage rates are fed there is increased total fecal excretion and the amount of fermentable solids within the feces also increases. A study found that stored manure from cattle fed a brown midrib variety of corn silage excreted 31% more fermentable solids in the feces and emitted 55% more methane from manure fermentation than manure from cattle fed conventional corn silage. Brown midrib varieties of forages are associated with being a higher quality forage due to their decreased indigestible NDF, improved NDF digestibility, and faster passage rates. Compared to the potential reductions in enteric methane described above, manure fermentation may offset benefits. Studies must simultaneously measure both enteric and manure fermentation as they relate to passage rate for true conclusions on net emissions to be drawn.

With mandates to reduce methane on dairy farms imminent, altering ruminal passage rate through forage selection and management may be a sustainable and effective way to reduce enteric emissions. Though, further studies are needed as animal studies specifically evaluating the influence of passage rate on enteric methane are scarce, and studies investigating its influence on manure fermentation are even more so. An economic analysis is also warranted to evaluate the impacts of increased dry matter intake and milk production from feeding rations with increased passage rates.

Of Note

The **Southeast Dairy Business Innovation Initiative** (SDBII) has opened applications for two grant opportunities and Virginia farms and producers are eligible. **Applications are due 3:00/EST on October 16, 2023.**

Learn more:

Specialty Processing Equipment Grant:
<https://sdbii.tennessee.edu/specialty-equipment-processing-grant/>

Dairy Business Planning Grant
<https://sdbii.tennessee.edu/dairy-business-planning-grant-open/>

→ **For assistance or more information please contact your local dairy agent.**

Upcoming Events

National 4-H Dairy Judging Contest
October 1, 2023
Madison, WI

World Dairy Expo
October 1-6, 2023
Madison, WI

2023 Stockmanship/Grazing Infrastructure Training

October 3-4, 2023

Weyers Cave

Dairy Calf Workshop

October 24, 2023

Rockingham County

National 4-H Dairy Quiz Bowl

November 5, 2023

Louisville, KY

Southern Regional Dairy Challenge

November TBA

Georgia

Hokie Dairy Day

December 2, 2023

Virginia Tech

VA WISE Cattle & Equipment

December 15-16, 2023

Grave's Mountain Lodge

If you are a person with a disability and require any auxiliary aids, services or other accommodations for any Extension event, please discuss your accommodation needs with the Extension staff at your local Extension office at least 1 week prior to the event.

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