Is This a Good Microbiome? What About That One? How Does the Microbiome Affect Efficiency and Productivity of My Herd?

W. Huang, J. M. Lourenco, T. D. Pringle, O. Y. Koyun, and T. R. Callaway
Department of Animal and Dairy Science
University of Georgia, Athens, GA

Introduction

Most of what we "know" about the microbial ecosystem of the rumen and the gut of cattle comes from correlative studies based on end-products and animal performance, but largely the function of the microbial ecosystem has remained a "black box" (Krause et al., 2013). There has been a revolution in the past 15 years in terms of information from Next Generation Sequencing (NGS) that has opened the composition and inner workings of the microbial population of the gut in ways that could never be imagined by Hungate or Bryant (Dowd et al., 2008, Callaway et al., 2009, Callaway et al., 2010, Lourenco et al., 2019, Lourenco et al., 2020, Welch et al., 2021). The ability to visualize the microbiome has enabled us to link specific bacteria (or fungi, or protozoa) to specific outcomes in a way that we can finally understand which microbes are most beneficial to the host or are selected for by diet or treatment. However, in many ways, this new power has been wielded like a child with a found handgun; pointed randomly to little purpose but making a loud noise. Instead of adding antibiotics to a diet and expecting "something good" to happen in terms of production response, but not understanding how (Pennycook and Scanlan, 2021); we can now determine which ecological factors impact the composition and degradative activity of the microbial population (Moraïs and Mizrahi, 2019, Grieneisen et al., 2021). As we further our understanding of how the microbiome functions in the real world, we can begin to make directed/targeted changes in the microbial population that can directly impact the animal.

Symbiosis: The Ruminant Gut

The symbiotic relationship between the ruminant animal and the resident microbial ecosystem of the gastrointestinal tract is unique and allows the ruminant animal to thrive on diets that monogastrics cannot (Hungate, 1944, 1947, Bryant and Burkey, 1952, Bryant, 1959, Bryant and Robinson, 1962, Hungate, 1966). The presence of this complex resident microbial consortium of bacteria, protozoa, and fungi gives the ruminant adaptability to utilize a wide variety of feedstuffs; however, this comes at a cost of feed efficiency, and in modern terms, reduced environmental sustainability. The anaerobic environment of the gut means the microbial population must depend upon the process of fermentation which produces the volatile fatty acids which is utilized by the host, but also H_2, CO_2, and CH_4 which are not utilized by the host animal. Methane (CH_4) is a greenhouse gas, but also represents a loss of carbon and energy from the ration that could be used for growth or milk production (McAnally and Phillipson, 1944, Johnson and Johnson, 1995, Boadi et al., 2004, Wright et al., 2004). Thus, the

fermentation characteristics clearly impact the host animal's physiological status, including fetal growth, lactation, and milk composition (Weimer et al., 2017). Moreover, the microbial population will change during the life of the cow from weaning through breeding and will also change dramatically with dietary shifts during the production cycle (Krause et al., 2020, Welch et al., 2020, Welch et al., 2021).

We have long recognized the gastrointestinal microbial population as a great biochemical reservoir of degradative activity (Figure 1), but the relationships between the individual microbes and their substrates, fermentation pathways, affinities, and end-products remain largely unknown except for a few well-studied species (e.g., *Ruminococcus*, *Streptococcus*) who are involved in fiber and starch fermentation (Ransom-Jones et al., 2012, Bandarupalli, 2017, Seshadri et al., 2018, Henderson et al., 2019). Next Generation Sequencing now allows us to "see" the composition of a large microbial population at once, so that instead of measuring what we consider to be "key" or "important" species we can determine the actual keystone organisms in real world conditions (Thomas et al., 2017). For instance, the presence of *Ruminococcus* populations were linked with beef cattle growth efficiency from weaning throughout the backgrounding and feedlot period (Krause et al., 2020, Welch et al., 2020, Welch et al., 2021). This technology allows us to begin asking questions about which specific microbial organisms are important and which are linked with increased milk production or altered body composition.
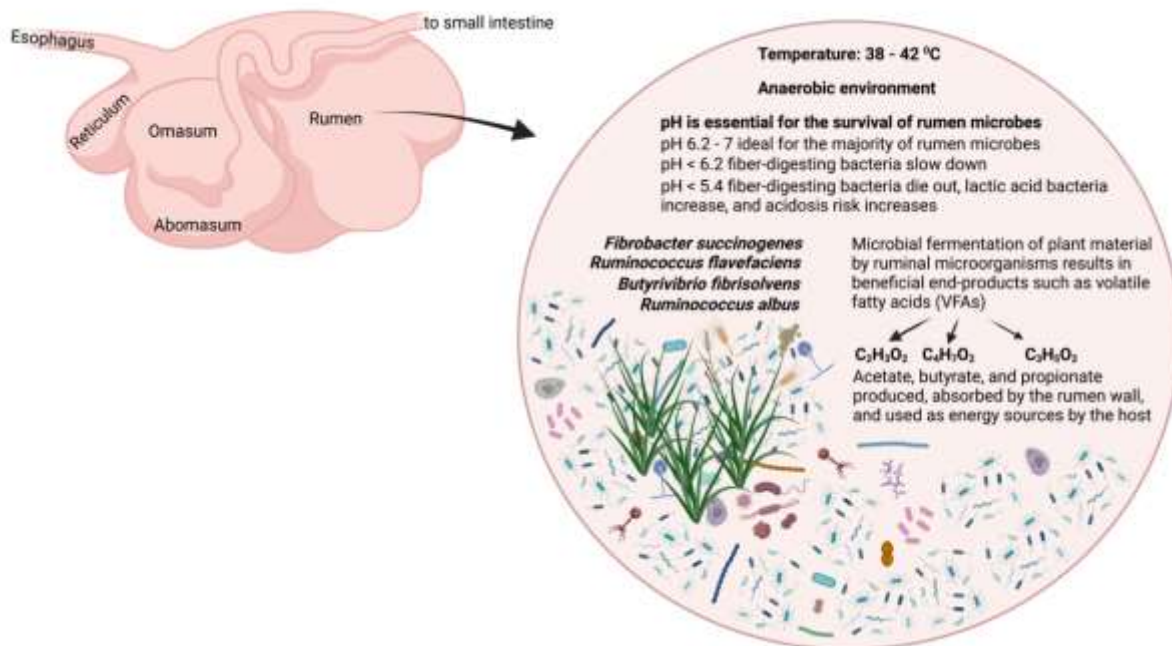

Figure 1. Role of the ruminal microbial population fermentation on ruminant nutrition.

Changes in the Gastrointestinal Microbial Ecosystem

The specific end-products of the gastrointestinal microbial fermentation vary based upon diet, and most especially upon energy density of the ration. We understand the impact of starch feeding on lactate production leading to acidosis (or subacute

acidosis) in our highest producing cows; but we have not demonstrated how starch impacts the microbial population of the gut in relation to the diet. While we do know that propionate is a primary end-product of ruminal starch fermentation, it results in a certain degree of milkfat depression (Hook et al., 2011). However, increasing acetate production from forage feeding to enhance milkfat production, results in increased methane production, which is a potent greenhouse gas and represents a significant loss of carbon and energy to the host cow (Hornung et al., 2018, Wallace et al., 2019, Bowen et al., 2020). Thus, we need to understand which organisms in the microbial population are linked with production of each of these important short chain fatty acids to manipulate the ruminal fermentation to meet the goals of your specific producers.

As calves mature, the microbiome changes throughout the gut and include the calf becoming a functional ruminant animal (Welch et al., 2021). Studies following beef calves from weaning through backgrounding and into the feedlot have found increasing microbial diversity in the rumen and hindgut as calves age (Krause et al., 2020, Welch et al., 2020, Welch et al., 2021). Specific members of the microbial population have been identified that are linked with increased production efficiency (Feed:Gain), and with carcass quality (marbling) (Krause et al., 2020, Welch et al., 2020, Welch et al., 2021). The fecal populations of methane producing organisms (archaea; methanogens) were increased in feedlot steers that were less efficient, compared to the more efficient steers (Carmichael et al., 2022). These studies indicate that the specific composition of the microbial population inhabiting the gut can have profound impacts on cattle physiology and energetic status.

While we have used antibiotics for years to alter the end-products of the ruminal (and gastrointestinal) fermentation in animals, we have not understood how antibiotics work to improve food production efficiency. As we increasingly regulate the use of antibiotics in animal agriculture, we have got to replace their benefits with some alternatives. To do so, we must understand HOW these compounds impact (i) animal performance, (ii) fermentation characteristics, and (iii) the native microbial population. The use of NGS allows us to finally begin to understand these impacts, and how alternatives to antimicrobials (ATA) genuinely work. Afterwards, we can begin to understand how each ATA impacts the microbial population and resultant milk production. Once we understand how those linkages actually function (as opposed to theoretically), then we can develop specific strategies tailored for specific production stages, specific health challenges (e.g., hemorrhagic bowel syndrome), production goals (e.g., fluid milk or milk fat), or even down to individual farms.

The improved understanding of antibiotic action will allow us to fully understand what probiotic approaches (including eubiotics, prebiotics, organic acids, and postbiotics) accomplish in the gut in terms of microbial ecological impacts. Figure 2 demonstrates the hypothesized impacts of probiotic approaches. Most of the effects are derived from stimulating a native (or introduced in case of eubiotics) microbial population to produce antimicrobial proteins (AMPs) or short chain fatty acids (SCFAs). These end-products can inhibit opportunistic (or obligate) pathogens from inhabiting the mucus layer near the epithelium of the gut. Excluding pathogens from this proximate

layer can prevent pathogen entry to epithelial cells and prevent them instigating inflammation. When epithelial cells undergo inflammation, the proteins holding epithelial cells in close proximity become weaker, and this loosens up the tight junctions between cells, which can allow the passage of toxins and pathogens into the bloodstream which can have very deleterious impacts throughout the animal. Probiotic action is thought to prevent this pathogen proximity but can also modulate the immune system by stimulating dendritic cell "sampling" of the gut microbiota, altering T- and B-cell proliferation which affects downstream cytokine regulation. Interestingly, a recent study has shown that when probiotics were fed to high producing lactating Holstein cows, the probiotic altered the expression of more than 11,000 genes of these cows (Adjei-Fremah et al., 2017). The genes that were both up- and down-regulated were scattered across 87 different bovine metabolic pathways, many of which involved suppression of inflammation and growth hormone production (Adjei-Fremah et al., 2017). Further studies have demonstrated that probiotic feeding in cattle affects changes in the production of many of the B-vitamins that meet the cow's requirements (Vandana et al., 2013).
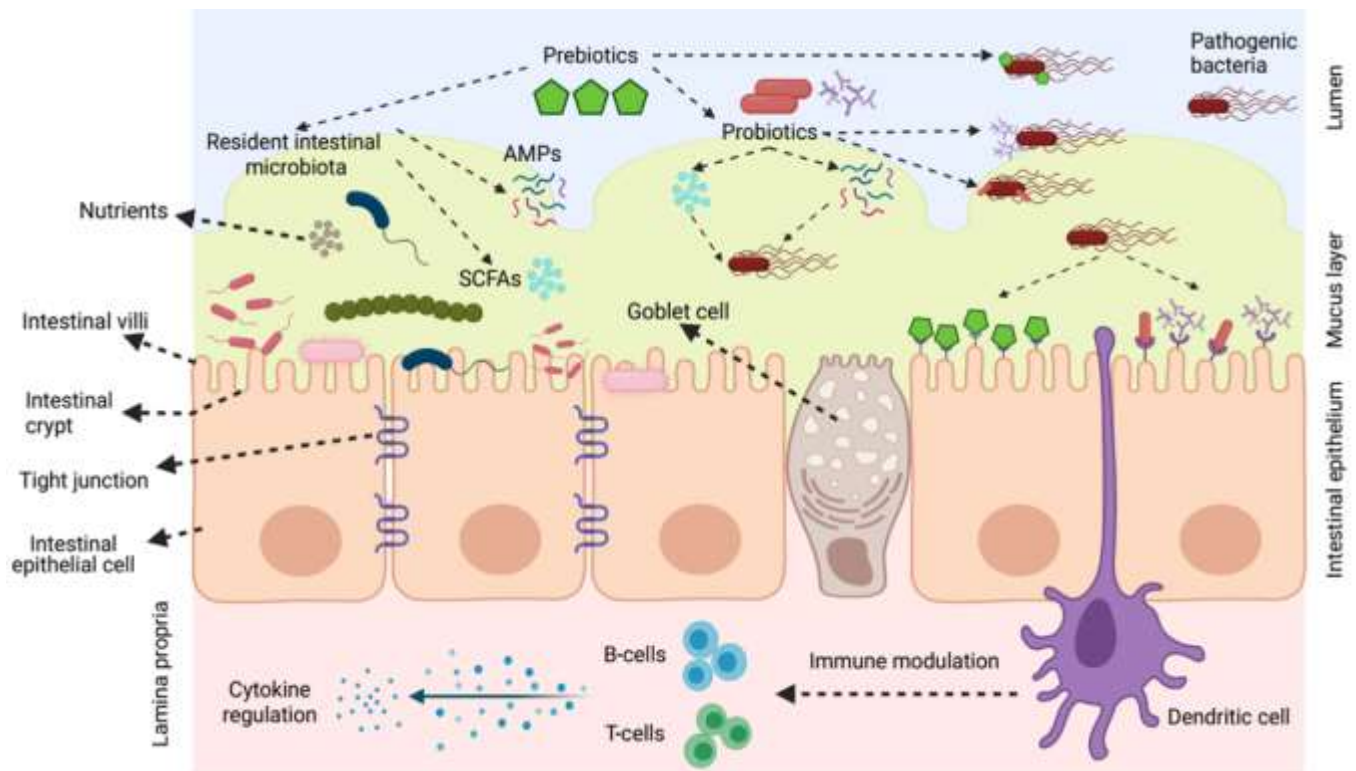

Figure 2. Modes of action of probiotic-type approaches to harness the power of the microbiome. An area, where we can now fully determine the impacts of both pro- and anti-biotic treatments, that offers opportunities to improve production efficiency.

Words of Caution

Being able to identify the microbial populations in detail has been intoxicating to microbial ecologists, and they have been tempted to use the microbiome analytic tools to answer all questions similarly to the axiom- "all the world looks like a nail when you

have a hammer". The temptation has been to perform microbiome analyses on all cattle in an undirected fashion, but without understanding the microbial activity or the nutrition and animal production factors this knowledge carries limited practical impact. We can look at a microbiome analysis like we would someone using a class photograph of elementary students to predict the impact of a group of children on society as adults. A class photo simply demonstrates the presence of absence of a member of the "class" on a specific day. This information in isolation has little value in our pursuit of societal-level impact, but when compiled over time/locations and other metrics aggregates value. Combining several metrics such as: continued daily attendance (which is often correlated with grades), discipline issues, grades, activities, clubs, internships, and goals; along with collegiate selection, work ethic, majors, personal ethics, activities, and jobs after college; begins to accumulate predictive value. Compiling this spectrum of "production" metrics can allow us to build models that are predictive of outcomes, both at an individual and societal level. So, by looking at the microbiome composition in cattle can tell us what is happening at that moment, but not much more. As we accumulate more information from multiple herds and conditions, including beef and dairy, we will be able to overlay a number of cattle over time, fed different diets, producing different milk fat and/or protein percentages, and different milk yields along with end-products (e.g., methane, VFA, ammonia, microbial crude protein). As this data accumulates, we can begin to construct a predictive model to harness the power of the microbiome.

Summary

Much like the new James Webb Telescope that allows us to see deeper into space, Next Generation Sequencing allows us to see deeper into the microbial world, including that within the gut of humans and food animals. The ability to understand which microbes are present in specific diets and production conditions, and which end-products they are linked to provides potential power to be able to predict outcomes. Clearly, the microbial population of the gastrointestinal tract impacts animal performance, efficiency, sustainability, animal health, and food safety. NGS allows us to be able to finally understand what probiotic and pathogen controlling approaches do to replace antibiotics and improve fermentation efficiency, production sustainability, production quality/quantity, animal health, and food safety. These new techniques offer the possibility of truly understanding how the ruminant microbial population works with the host animal to degrade feed and can allow us to simultaneously control production efficiency, end-products, and wasteful fermentations to improve sustainability and (more directly important to us) profitability of dairy production.

References

Adjei-Fremah, S., K. Ekwemalor, E. Asiamah, H. Ismail, S. Ibrahim, and M. Worku. 2017. Effect of probiotic supplementation on growth and global gene expression in dairy cows. J. Appl. Anim. Res. 46.

Bandarupalli, V. 2017. Identification of novel rumen bacteria using starch as a selective nutrient in batch cultures. J. Anim. Sci. 95:305.

Boadi, D., C. Benchaar, J. Chiquette, and D. Massao. 2004. Mitigation strategies to reduce enteric methane emissions from dairy cows: Update review. Can. J. Anim. Sci. 84(3):319-335.

Bowen, J. M., P. Cormican, S. J. Lister, M. S. McCabe, C.-A. Duthie, R. Roehe, and R. J. Dewhurst. 2020. Links between the rumen microbiota, methane emissions and feed efficiency of finishing steers offered dietary lipid and nitrate supplementation. PLOS ONE 15(4):e0231759.

Bryant, M. P. 1959. Bacterial species of the rumen. Bacterial Rev. 23:125-153.

Bryant, M. P. and L. A. Burkey. 1952. Numbers and some predominant groups of bacteria in the rumen of cows fed different rations. J. Dairy Sci. 36:218-224.

Bryant, M. P. and I. M. Robinson. 1962. Some nutritional characterisics of predominant culturable ruminal bacteria. J. Bacteriol. 84:605-614.

Callaway, T., S. Dowd, T. Edrington, R. Anderson, N. Krueger, N. Bauer, P. Kononoff, and D. Nisbet. 2010. Evaluation of bacterial diversity in the rumen and feces of cattle fed different levels of dried distillers grains plus solubles using bacterial tag-encoded FLX amplicon pyrosequencing. Journal of Animal Science 88(12):3977-3983.

Callaway, T. R., S. E. Dowd, R. D. Wolcott, Y. Sun, J. L. McReynolds, T. S. Edrington, J. A. Byrd, R. C. Anderson, K. N, and D. J. Nisbet. 2009. Evaluation of the bacterial diversity in cecal contents of laying hens fed various molting diets using bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP). Poult. Sci. 88:298-302.

Carmichael, M. N., J. M. Lourenco, C. B. Welch, D. B. Davis, T. R. Krause, M. J. Rothrock, F. L. Fluharty, T. D. Pringle, and T. R. Callaway. 2022. Ruminal Microbiome Differences in Angus Steers with Differing Feed Efficiencies During the Feedlot-Finishing Phase. Microorganisms:In Press.

Dowd, S. E., T. R. Callaway, Y. Sun, T. McKeehan, R. G. Hagevoort, and T. S. Edrington. 2008. Evaluation of the bacterial diversity in the feces of cattle using bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP). BMC Microbiol. 8:125-132.

Grieneisen, L., M. Dasari, T. J. Gould, J. R. Björk, J. C. Grenier, V. Yotova, D. Jansen, N. Gottel, J. B. Gordon, N. H. Learn, L. R. Gesquiere, T. L. Wango, R. S. Mututua, J. K. Warutere, L. Siodi, J. A. Gilbert, L. B. Barreiro, S. C. Alberts, J. Tung, E. A. Archie, and R. Blekhman. 2021. Gut microbiome heritability is nearly universal but environmentally contingent. Science 373(6551):181-186.

Henderson, G., P. Yilmaz, S. Kumar, R. J. Forster, W. J. Kelly, S. C. Leahy, L. L. Guan, and P. H. Janssen. 2019. Improved taxonomic assignment of rumen bacterial 16S rRNA sequences using a revised SILVA taxonomic framework. PeerJ 7:e6496.

Hook, S. E., M. A. Steele, K. S. Northwood, J. Dijkstra, J. France, A. D. G. Wright, and B. W. McBride. 2011. Impact of subacute ruminal acidosis (SARA) adaptation and recovery on the density and diversity of bacteria in the rumen of dairy cows. FEMS Microbiology Ecology 78(2):275-284.

Hornung, B., B. v. den Bogert, M. Davids, V. A. P. Martins dos Santos, C. M. Plugge, P. J. Schaap, and H. Smidt. 2018. The Rumen Metatranscriptome Landscape

Reflects Dietary Adaptation and Methanogenesis in Lactating Dairy Cows. bioRxiv:275883.

Hungate, R. E. 1944. Studies on cellulose fermentation. I. The culture and physiology of an anaerobic cellulose-digesting bacterium. J. Bact. 48:499-512.

Hungate, R. E. 1947. Studies on cellulose fermentation. III: The culture and isolation of cellulose-decomposing bacteria from the rumen of cattle. J. Bact. 53:631-644.

Hungate, R. E. 1966. The Rumen and its Microbes. Academic Press, New York, NY.

Johnson, K. A. and D. E. Johnson. 1995. Methane emissions from cattle. J. Anim. Sci. 73(8):2483-2494.

Krause, D. O., T. G. Nagaraja, A. D. G. Wright, and T. R. Callaway. 2013. Rumen microbiology: Leading the way in microbial ecology. J. Anim Sci. 91:331-341.

Krause, T. R., J. M. Lourenco, C. B. Welch, M. J. Rothrock, T. R. Callaway, and T. D. Pringle. 2020. The relationship between the rumen microbiome and carcass merit in Angus steers. J. Anim. Sci. 98(9):skaa287.

Lourenco, J. M., T. R. Callaway, T. J. Kieran, T. C. Glenn, J. C. McCann, and R. L. Stewart. 2019. Analysis of the Rumen Microbiota of Beef Calves Supplemented During the Suckling Phase. Frontiers in Microbiology 10(1131).

Lourenco, J. M., T. J. Kieran, D. S. Seidel, T. C. Glenn, M. F. D. Silveira, T. R. Callaway, and R. L. Stewart, Jr. 2020. Comparison of the ruminal and fecal microbiotas in beef calves supplemented or not with concentrate. PLoS One 15(4):e0231533.

McAnally, R. A. and A. T. Phillipson. 1944. Digestion in the ruminant. Biological Reviews 19(2):41-54.

Moraïs, S. and I. Mizrahi. 2019. Islands in the stream: from individual to communal fiber degradation in the rumen ecosystem. FEMS Microbiology Reviews 43(4):362-379.

Pennycook, J. H. and P. D. Scanlan. 2021. Ecological and Evolutionary responses to Antibiotic Treatment in the Human Gut Microbiota. FEMS Microbiology Reviews 45(5).

Ransom-Jones, E., D. L. Jones, A. J. McCarthy, and J. E. McDonald. 2012. The Fibrobacteres: An Important Phylum of Cellulose-Degrading Bacteria. Microb. Ecol. 63(2):267-281.

Seshadri, R., S. C. Leahy, G. T. Attwood, K. H. Teh, S. C. Lambie, A. L. Cookson, E. A. Eloe-Fadrosh, G. A. Pavlopoulos, M. Hadjithomas, N. J. Varghese, D. Paez-Espino, c. Hungate project, N. Palevich, P. H. Janssen, R. S. Ronimus, S. Noel, P. Soni, K. Reilly, T. Atherly, C. Ziemer, A.-D. Wright, S. Ishaq, M. Cotta, S. Thompson, K. Crosley, N. McKain, R. J. Wallace, H. J. Flint, J. C. Martin, R. J. Forster, R. J. Gruninger, T. McAllister, R. Gilbert, D. Ouwerkerk, A. Klieve, R. A. Jassim, S. Denman, C. McSweeney, C. Rosewarne, S. Koike, Y. Kobayashi, M. Mitsumori, T. Shinkai, S. Cravero, M. C. Cucchi, R. Perry, G. Henderson, C. J. Creevey, N. Terrapon, P. Lapebie, E. Drula, V. Lombard, E. Rubin, N. C. Kyrpides, B. Henrissat, T. Woyke, N. N. Ivanova, and W. J. Kelly. 2018. Cultivation and sequencing of rumen microbiome members from the Hungate1000 Collection. Nature Biotechnology 36:359.

Thomas, M., M. Webb, S. Ghimire, A. Blair, K. Olson, G. J. Fenske, A. T. Fonder, J. Christopher-Hennings, D. Brake, and J. Scaria. 2017. Metagenomic

characterization of the effect of feed additives on the gut microbiome and antibiotic resistome of feedlot cattle. Scientific Reports 7(1):12257.

Vandana, R., Y. Brijesh, and G. P. Lakhani. 2013. Application of probiotic and prebiotic in animals production: a review. Environment and Ecology 31(2B):873-876.

Wallace, R. J., G. Sasson, P. C. Garnsworthy, I. Tapio, E. Gregson, P. Bani, P. Huhtanen, A. R. Bayat, F. Strozzi, F. Biscarini, T. J. Snelling, N. Saunders, S. L. Potterton, J. Craigon, A. Minuti, E. Trevisi, M. L. Callegari, F. P. Cappelli, E. H. Cabezas-Garcia, J. Vilkki, C. Pinares-Patino, K. O. Fliegerová, J. Mrázek, H. Sechovcová, J. Kopečný, A. Bonin, F. Boyer, P. Taberlet, F. Kokou, E. Halperin, J. L. Williams, K. J. Shingfield, and I. Mizrahi. 2019. A heritable subset of the core rumen microbiome dictates dairy cow productivity and emissions. Science Advances 5(7):eaav8391.

Weimer, P. J., M. S. Cox, T. Vieira de Paula, M. Lin, M. B. Hall, and G. Suen. 2017. Transient changes in milk production efficiency and bacterial community composition resulting from near-total exchange of ruminal contents between high- and low-efficiency Holstein cows. J. Dairy Sci. 100:7165-7182.

Welch, C. B., J. M. Lourenco, D. B. Davis, T. R. Krause, M. N. Carmichael, M. J. Rothrock, T. D. Pringle, and T. R. Callaway. 2020. The impact of feed efficiency selection on the ruminal, cecal, and fecal microbiomes of Angus steers from a commercial feedlot. J. Anim. Sci. 98(7).

Welch, C. B., J. M. Lourenco, T. R. Krause, D. S. Seidel, F. L. Fluharty, T. D. Pringle, and T. R. Callaway. 2021. Evaluation of the Fecal Bacterial Communities of Angus Steers With Divergent Feed Efficiencies Across the Lifespan From Weaning to Slaughter. Frontiers in veterinary science 8(694).

Wright, A. D. G., P. Kennedy, C. J. O'Neill, A. F. Toovey, S. Popovski, S. M. Rea, C. L. Pimm, and L. Klein. 2004. Reducing methane emissions in sheep by immunization against rumen methanogens. Vaccine 22(29-30):3976-3985.