

Title:

Breeding for the Little Things: A Look at Including Microbiome Information in Animal Breeding

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Biographical sketch (please do not exceed 250 words):

Andrew Lakamp is from Central Illinois and is the son of Jeff and Debbie Lakamp. Drew grew up showing Shorthorn cattle. At 12, he stated he would be a doctor of genetics so he could “breed the best show cows.” Now, more than 15 years later, Drew has obtained a bachelor's degree in animal science from Southern Illinois University Carbondale, a master's in animal breeding from Kansas State University, and is working on his doctorate in animal breeding and genetics from the University of Nebraska-Lincoln. Drew hopes to become a professor of animal science at a university where he can help improve the beef industry through research, extension, and classroom education.

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I certify that I have read and had sufficient time to provide feedback on the attached literature review.



Advisor Signature Date 4/12/2024

1 Introduction

In the recent past, animal genomic data was the disruptive technology that faced animal breeding. New methodologies had to be developed to incorporate the valuable information genomics could provide. Now, following the improvement in sequencing technology, the microbiome is echoing that same trajectory. The microbiome is the collection of microorganisms that live in or on an animal. While the term microbiome can be used to refer to all the bacteria, archaea, protists, and fungi that live symbiotically with an animal, it can also be used in a site-specific manner, i.e., the rumen microbiome. The microbiome has been shown to be associated with traits important to livestock production from body weight and gain to health and fertility (Wang et al., 2023; Aliakbari et al., 2022; Ramayo-Caldas et al., 2021; and Sanglard et al., 2019, respectively). Various forces drive the composition of the microbiome, but the one most relevant to animal breeders is the host genetic factor (Ryu and Davenport, 2022). Thus, microbiomic information can be useful to animal breeders in a multitude of ways. First and foremost, microbiome data offers a wealth of new phenotypes available for genetic selection. Consider, for example, that the cattle genome contains roughly 22,000 genes (The Bovine Genome Sequencing and Analysis Consortium et al., 2009) compared to the 13.8 million unique prokaryotic genes found in the bovine rumen microbiome (Li et al., 2020). By selecting for or against certain microbial populations, breeders could improve animal health and production while also lowering the animal's carbon footprint. As a corollary, microbial traits could be utilized in a multi-trait model to improve the breeding value estimation of harder to measure traits, such as methane production. Secondly, microbiomic information could be included in a selection index to parse out and optimize selection on changes in metabolic pathways in the animal versus beneficial changes resulting from the host's effect on the microbiome. Lastly, information from the microbiome can be used in concert with genomic information to improve both genetic and phenotypic predictions of animals for economically important traits.

2 Review of Literature

2.1 Review of Microbiome Data

Like genomic data, microbiomic data can be generated in a number of ways. The two most common methods are targeted sequencing and whole metagenome shotgun (WMS) sequencing.

In targeted sequencing, a specific region of DNA is targeted with a primer, amplified, and sequenced. The 16S ribosomal ribonucleic acid (16S rRNA) gene is the most common target gene (Pace et al., 1986). The properties of this gene allow for straightforward clustering and, if available, taxonomical identification. The primary benefits of targeted sequencing are the relatively low cost, established bioinformatics pipelines, and large databases for reference (Ranjan et al., 2016). Nonetheless, the drawbacks to amplicon sequencing are worth considering. Analyses using 16S rRNA are very limited. Only prokaryotic DNA is sequenced, and then only the presence and relative abundance of the taxa is documented. No functional genes can be directly measured.

In direct opposition to targeted gene sequencing is whole metagenome shotgun sequencing. In general, the data generation process between the two methods is similar. The key difference is that no specific gene is amplified for sequencing (Quince et al., 2017). In other words, rather than amplifying a specific gene, WMS sequencing attempts to sequence all the DNA in a sample. The WMS sequencing method generates a lot of data, up to 1.5 Tb per run (Quince et al., 2017). This improves species detection compared to target gene methodologies and provides information about the abundance of microbial genes (Ranjan et al., 2015; Ranjan et al., 2016); however, it is very computationally demanding. In addition, WMS sequencing is not constrained to prokaryotic organism detection, although this can be a double-edged sword. While WMS sequencing can be used to gather information about eukaryotic microbes, it also requires that contamination (sequence reads from other sources other than the microbiome, i.e., human, bovine, etc.) to be accounted for in the resulting data (Quince et al., 2017). Lastly, the largest logistical restraint on WMS sequencing is the price compared to targeted sequencing (Quince et al., 2017).

Both sequencing methods, targeted and WMS sequencing, will result in data that can be used in various ways by animal breeders. However, slightly more preparatory work needs to be conducted before microbiomic data can be used to its full potential. The data generated are sequence reads and not every animal is sequenced to the same depth. Thus, the reads are standardized, usually by taking the relative abundance, which is the ratio of counts of a microbial feature to the total read count for that sample (Tsilimigras and Fodor, 2016). Further, those

relative abundances are often transformed (e.g., \log_{10}) so each feature approximates a normal distribution over the population and the assumptions of classical statistical tests remains intact.

In conclusion, there are many forms microbiomic data can take. Targeted gene sequencing allows a snapshot of the prokaryotic life in a microbiome, while a more extensive portrait can be garnered from whole metagenomic sequencing. Like genomics, microbiomics has sparked a vast array of new methods to analyze the information available. In general, though, the \log_{10} transformed relative abundance of a microbial feature is often the phenotype used in conjunction with genomic data. This relatively simple adjustment to the raw data allows for direct comparison between samples with more common statistical tools for ease of analysis.

2.2 Microbiability

In animal breeding the heritability of a trait is quantified as the ratio of additive genetic variance to phenotypic variance. In the same way, the ratio between microbial variance and phenotypic variance can be calculated. This value has been coined “microbiability” (m^2).

The idea of microbiability was originally proposed by Difford et al. (2016) but later expanded upon by multiple authors (Difford et al., 2018; Camarinha-Silva et al., 2017). An illustration of the idea can be seen as the Microbiome model of Figure 1. The estimation of microbiability is almost identical to any method of estimating heritability using single nucleotide polymorphism (SNP) data. The difference being instead of SNP counts, microbial information is used. For example, a microbial relationship matrix (MRM) can be created using normalized microbial relative abundances then implemented in a similar fashion as a genomic relationship matrix would be used in a mixed model framework (Ross et al., 2013; Camarinha-Silva et al., 2017). From there, a population parameter of microbiome variance can be estimated. Further, instead of an expected breeding value (EBV), this substitution results in estimated microbiomic values (EMV). Here the EMV of an animal is the deviation in performance of that animal from the population average due to its microbial composition, rather than its genetics (González-Recio et al., 2023).

Microbiability estimates are scarce for beef cattle. Though, there are estimates in other species that indicate the microbiome influences a large variety of traits. In dairy cattle, a microbiability estimate (standard error) for methane was 0.13 (0.08) (Difford et al., 2018). Zang

et al. (2022) created two MRM, one based on data from heritable microbial organisms (h^2 different from 0 at $P < 0.05$) and another based on non-heritable microbial data. From these the authors calculated microbiability estimates for lactation traits using both the heritable and non-heritable microbiota. The heritable microbiota generally resulted in low microbiability estimates ranging from 0.05 to 0.11 (milk fat and milk yield, respectively) while the non-heritable microbiota resulted in estimates from 0.20 to 0.39 (energy-corrected milk and milk yield, respectively). In sheep, 180-day weight had microbiability estimate of 0.20 (0.06) (Wang et al., 2023). Comparatively, microbiability of 8-month liveweight were 0.31 (0.13), 0.39 (0.18), and 0.43 (0.16) for grass-fed lambs, grass-fed adults, and lucerne-fed lambs, respectively (Hess et al., 2023). The rough concordance of the microbiability estimates between the two papers is interesting, especially considering the studies used different populations and different sequencing techniques. One of the most popular MRM creation method comes from Camarinha-Silva et al. (2017). These authors also reported the first microbiability of production traits in livestock, focusing on swine. The estimates for average daily gain, feed conversion, and feed intake based on 207 Pietrain sows were 0.28 (0.13), 0.21 (0.14), and 0.16 (0.10), respectively. In Large White pigs, the microbiability for the same production traits was lower according to Aliakbari et al. (2022). Here the estimate for average daily gain was 0.05 (0.05), feed conversion was 0.22 (0.11), and feed intake was 0.06 (0.06). The authors additionally provided estimates of the microbiability of residual feed intake at 0.12 (0.09) and backfat at 0.11 (0.06). Interestingly, the impact of the gut microbiome is not limited to methane and feed-related production traits. Ramayo-Caldas et al. (2021) reported the microbiability for IgM, IgG, and 19 other indicators of immunocompetence was at least 0.15 and up to 0.28 in swine. Lastly, the MRM need not be limited to gut microbiomes. The vaginal microbiome can also be sampled for its microbiota and the same methods for estimating microbiability can be applied. Though, at least in relation to farrowing performance the impact is extremely low ($m^2 < 0.01$) for most traits (Sanglard et al., 2019).

Unlike heritability, estimates of microbiability can change given the microbiome of an animal is dynamic. While the microbiome may be resilient and have its own sort of inertia (Weimer, 2015), we know it fluctuates over the course of an animal's life (Jami et al., 2013), over the course of a day (Shaani et al., 2018), and especially with diet (Calsamiglia et al., 2008). Thus, any microbiome sample is a snapshot of that microbiome at that time and any microbiability

estimate reflects that. Therefore, it holds that microbiability estimates should be higher for traits recorded closer in time to when the microbiome was sampled. This is supported by Wang et al. (2023) who reported the microbiability, derived using data from a sample taken at 180-days-old, was nonsignificant for birthweight, and then roughly 0.20 for bodyweight taken every 20 days from 100 days of age to 180 days of age. Secondly, though the methodology to create an MRM developed by Camarinha-Silva et al. (2017) is the most common in literature, it is certainly not the only option available. Different methods of creating the MRM can lead to drastically different estimates of microbiability. Saborío-Monero et al. (2021) used simulated data to set microbiability to 0.30 and created 12 different MRM, each using a different method that is commonly used to create a (dis)similarity matrix. The estimates from each of these matrices ranged from 0.27 to 0.97. Additionally, Hess et al. (2020) showed that microbiability estimates differ with type of sequencing information used, even if the population, trait, and modelling procedure remain static. In the same way an animal can have both a mitochondrial and nuclear genome, an animal can have several different microbiomes in and on its body (e.g., rumen, ocular, nasal, skin, etc.). Though no study has compared the microbiability estimates of the same trait from different microbiomes on the same animal, it is still a source of variability between estimates. Finally, we know that the host's genetics influence their microbiome's composition. One could postulate that if host genetics were fit in a model alongside microbial information, microbiability estimates would decrease. Interestingly, this does not seem to be the case as microbiability estimates for production traits in swine and liveweight in sheep only see a very small decrease (Aliakbari et al., 2022; Wang et al., 2023).

2.3 Microbiota as Selection Criteria

The log-transformed relative abundance of a microbial feature (or suite of features) can be used as selection criterion in order to aid genetic progress of economically important traits. This tactic is known as a microbiome-driven breeding strategy. For a microbiome-driven breeding strategy to be successful, the microbial trait must be present in much of the population (i.e., part of the core microbiome), be phenotypically variable, be heritable, and be genetically correlated with the trait of interest (González-Recio et al., 2023). See the Indirect model in Figure 1 for a visual representation of a microbiome-driven breeding value.

Of the various microbiomes in and on beef cattle and other ruminants, the most studied and a likely place to find microbial traits that meet the criteria for a microbiome-driven breeding strategy is the rumen. The rumen is home to an almost incalculable number of interacting microbiota and supplies the ruminant with up to 70% of the animal's protein and energy needs (Flint, 2005). Various rumen microbial features have been shown to be heritable in dairy cattle with estimates from 0.08 to greater than 0.7 (Sasson et al., 2017; Wallace et al., 2019; Saborío-Monero et al., 2020), in beef cattle with an estimate range of 0.06 to 0.82 (Abbas et al., 2020; Li et al., 2019), and in sheep with the heritability of genus-level abundances ranging from 0.05 to 0.34 (Hess et al., 2023). While operational taxonomic unit (OTU) or genus-level abundances were the most common trait analyzed in these studies, Martínez-Álvaro et al. (2022) showed microbial gene abundances from the rumen of beef cattle were also heritable (0.21 - 0.61). Thus, a variety of microbial feature abundances have achieved three of the four criteria to be considered as selection candidates. The filtering protocols of these studies restricted the features to those with high presence across the population. On average, only features present in at least 28.59% of samples were analyzed; however, prevalence ranged from 1% (Abbas et al., 2020) to 70% (Saborío-Monero et al., 2020). These features have non-zero heritability estimates, which also implies phenotypic variance across the population. The last item to consider is the genetic correlation of these features to economically important traits.

Unfortunately, there is limited information on correlations between microbial features and the other traits in beef cattle. Some research, such as that done by Li et al. (2019), indicates some SNP are associated with both microbial features and an economically important trait like feed efficiency, but fail to report any correlations. Martínez-Álvaro et al. (2022) is one of the few who reported correlations. Here, the authors found that additive log ratio of 29 microbial genera and that of 115 microbial genes were genetically correlated with methane production ($P < 0.05$). Those correlations ranged from -0.90 to 0.85. Moreover, using the equation for correlated response revealed the response to selection on 30 microbial gene abundances with strong genetic correlations to methane was estimated to decrease methane production 22%-34% more effectively than direct selection on methane production, depending on selection intensity. This represents a unique case where indirect selection is more efficient, and practically the trade-offs in costs of data collection and thus data density would also need to be considered.

Other animals may provide additional insight in how microbes might be used as selection criteria. Saborío-Montero et al. (2019) reported genetic correlations between the composition of microbial genera and methane production to range from -0.76 to 0.65 in dairy cattle. Moreover, the authors were able to identify a suite of seven genera whose relative abundance showed moderate heritability estimates (0.28 - 0.32) as well as positive genetic correlations with methane production (0.43 - 0.56). While not ruminants, genetic correlations have been documented between the log-transformed relative abundance of 22 fecal genera and feed efficiency, feed intake, growth, and backfat in swine (Aliakbari et al., 2021). Likewise, genetic correlations were found between a variety of fecal OTU and the average daily gain of rabbits (Mora et al., 2022).

In short, these authors have identified candidates to build a microbiome-driven breeding strategy to reduce methane emissions, increase production, or improve efficiency. The implications of this are two-fold. Firstly, selection of microbiome traits can indirectly improve hard to measure traits like methane production. Secondly, adding microbiome traits into a multivariate model, even if not chosen as selection criteria, will improve breeding value estimation of the trait of interest if data are available before the trait of interest can be observed.

2.4 Microbiome Traits in a Selection Index

The fact that aspects of an animal's microbiome can influence its phenotype and that an animal's genotype can influence aspects of its microbiome has been established in the scientific literature, and this body of evidence continues to grow. Moreover, it is very well established that an animal's genotype influences its phenotype. Consequently, the challenge becomes how to best untangle all these effects from each other and use the information available for selection most effectively. Several authors have proposed using a recursive structural equation model as one potential solution (Saborío-Monero et al., 2020; Christensen et al., 2021; Tiezzi et al., 2021). This approach would account for the covariance between the host genetic influence directly on the phenotype of interest and the genetic influence mediated by the microbial feature (or indirect influence). In other words, this procedure would separate the host genes that affect the phenotype directly from the host genes that affect the microbiome which then affect the phenotype (Recursive model in Figure 1). Thus, the total breeding value for the phenotype would be the direct genetic effect plus the sum of mediated genetic effects (Tiezzi et al., 2021; Christensen et al., 2021).

Parsing the genetic influence on a trait in a recursive structure can have benefits. From a biological perspective it provides new insight into causality (Valente et al., 2010) and aids in identifying novel quantitative trait loci (Tiezzi et al., 2021). From a breeding perspective, these two genetic influences can be split and weighted in a selection index based on the breeder's preferences (Weishaar et al., 2019). This could be of extreme value in making desired changes in one phenotype and either reduced or null antagonistic changes in another. For example, the genetic correlation between the first principal component (PC) of microbial composition and methane production in dairy cattle is 0.83 (0.13), while the genetic correlation between that same PC and dry matter intake was 0.32 (0.36) (López-Paredes et al., 2021). Compare this to the genetic correlation of 0.83 between methane production and feed intake in cattle (Donoghue et al., 2016; Manzanilla-Pech et al., 2016). Thus, if one were to select for cattle with lowered methane production but wanted to limit the impact of selection on feed intake, a selection index heavily favoring the microbiome mediated genetic effect on methane production might accomplish that goal. While methane is a good example and one of the very few that have reported genetic correlations between the microbiome and other traits, it is by no means the only trait that may benefit from this approach.

2.5 Predicting Phenotypes

There are many situations in which one might want to predict an animal's phenotype to inform management decisions. As has been established, microbiomic data can be treated similarly to genomic data, albeit with slight modifications to account for the differences in data format. It has also been established that aspects of the microbiome affect an animal's phenotype. Therefore, it is reasonable to attempt to predict phenotypes using microbiomic data with methods adopted from genomics. Outside of the potential benefits of predicting phenotype, fitting microbiome data into genetic prediction models has the potential to reduce the residual variation, this leading to more accurate genetic prediction models.

One of the more popular methods to predict phenotypes using microbiome information is to use the MRM in a best linear unbiased prediction (BLUP) context. This method substitutes an animal's genetic effect for the animal's microbiome effect by defining the relationship between animals through an MRM rather than a genetic-based relationship matrix. This idea was first presented in Ross et al. (2013). In that work, the microbiome of dairy cattle was used to predict

methane production. The authors had access to 5 different populations ($N = 7 - 20$) with both microbiomic and methane data. The experiment was constructed such that one population served as the training set for one other population in all pairwise combinations. Accuracy was defined as the Pearson correlation between observed and predicted phenotype and ranged from 0 to 0.79. These results demonstrate, much like genomic prediction, microbiomic phenotype prediction can be predictive, but the accuracy is dependent on the composition of the training and test populations.

Ross et al. (2013) explored phenotype prediction using only microbial information, however, other authors explored the differences between predicting phenotypes using genomics and microbiomics. The basic framework for these papers was to compare prediction accuracies between models with a random animal genetic effect, a random animal microbiome effect, and a model with both with no interaction (the additive approach or the Joint model in Figure 1). Wang et al. (2015) predicted residual feed intake phenotypes for 28 dairy cattle. The correlation between the predicted and observed values using just genomic information for all animals was 0.33. In a leave-one-out validation, the correlation using just rumen microbiomic data was 0.49 whereas the correlation was 0.57 when both types of information were included. Aliakbari et al. (2022) applied this structure to performance traits in pigs using 20-fold cross-validation. While the accuracies of predicted phenotype with adjusted phenotype varied across trait, overall, the accuracy of genomic prediction was comparable to or slightly higher than the microbiome predictions. However, the combination of the two information sources produced the most accurate predictions. Further examples of the greater predictive power of using both genomic and microbiomic data can be found in dairy cattle methane production and various lactation traits (Qadri et al., 2022) and sheep methane production, liveweight, and fecal egg count (Ross et al., 2020; Hess et al., 2021; Hess et al., 2023).

It has been established that the microbiome and host genetics are not totally independent. This overlap may account for why there are the accuracy gains from using genomic and microbiomic data are not the additive gains one might expect from two separate sets of predictors. Methods have been developed on how to account for this interaction. Saborío-Monero et al. (2021) utilized a linear model in a BLUP framework which included the animal genetic effect, the animal microbiome effect, and the interaction between the two where the (co)variance

matrix was defined as the Hadamard product between the GRM and the MRM. The correlation between the solutions for the interaction effect, what the authors termed estimated genetic \times microbiome interaction value (EGMV), and methane production in dairy cattle was 0.97. This outperformed models where EBV was the only solution (a standard GBLUP) which had accuracy of 0.9. The interaction solution also had better prediction than the EBV and EMV solutions from an additive model when the latter solutions were estimated together, but used to predict independently (accuracy of 0.86 and 0.81, respectively)

In another study, Qadri et al. (2022) compared models that used genomics only, microbiomics only, the additive approach, and two interaction models. One interaction model contained a Hadamard product and the other defined the interaction using a matrix derived from the Cholesky decomposition of the MRM and GRM known as CORE-GREML (Zhou et al., 2020). When predicting a suite of lactation and methane emission traits in Holsteins, the Hadamard interaction model always resulted in the greatest prediction accuracies. The extent this was the case depended on the trait in question. The models were then used to predict daily gain, feed intake, and feed conversion of the 207 pigs from Camarinha-Silva et al. (2017). For those traits, the Hadamard product model was the most accurate for feed conversion, competitive for daily gain and feed intake (slightly behind the additive model and microbiomic model, respectively).

There are methods other than the BLUP approach to predict phenotypes using microbiomic information. For example, Maltecca et al. (2019) contrasted the performance of models including Bayesian Lasso, random forest, gradient boosting, and semi-parametric kernel (Reproducing Kernel Hilbert space). The data utilized were microbiome samples from three timepoints (18 days of age, 118 days of age, and 196 days of age). Phenotypes comprised of loin depth, loin eye area, backfat thickness, weight taken roughly 110 days of age, 196 days of age, and at finish. Lastly, ADG was measured from birth to 110 days of age, 110 to 196 days of age, and 110 days of age to finish. Each phenotype/timepoint was subjected to a stratified 5-fold cross-validation with a 30% test set for each model. In general, sampling time played a critical role in determining prediction accuracy over a fixed effects model (the Null model in Figure 1). Microbiome data showed the greatest prediction improvement when the sample was taken closer to the recording of the phenotype. There was no clear winner between the models. All of the

models showed improvement over a fixed effect model for at least some phenotype/timepoint. The Reproducing Hilbert Kernel space model was the most consistent across phenotype/timepoint.

Regardless of the models used, microbiomic data can be useful for phenotypic prediction. Moreover, when used in concert with genomic information, either in an additive fashion and especially as an interaction, the prediction accuracy is greater than either information source alone. It is worth noting that while the genotypes of an animal will remain static throughout an animal's life, the same cannot be said of microbiomic features and their relative abundances. Those are most useful when taken close in time to the measurement of the phenotype of interest. Nonetheless, the optimal timing of microbiome sampling and the limits of early sampling require additional research.

3 Conclusions and Implications to Genetic Improvement of Beef Cattle

Microbiome data is a useful source of information whose full potential is only now becoming uncovered. It is being generated more commonly in research settings and more cheaply for all livestock species. The microbiome itself can provide useful insights into an animal's health and well-being in addition to being altered to optimize efficiency. Its use in animal breeding is multi-faceted, and several approaches have been proposed to make use of these data in genetic/phenotypic prediction models.

Microbial features can be used as phenotypes themselves. By directly selecting individual microbial features related to economically important traits, breeders have a new avenue with which to make improvements. For some economically important traits, selection on a suite of microbial features may result in more response to selection than direct selection.

Microbial features can be added in multivariate models as a correlated trait to increase EBV accuracy. Alternatively, a recursive model can be used to separate the direct and mediated influence of animal's genetics. By placing different selection emphasis on these partitions, breeders could make genetic gain in a trait while mitigating antagonistic changes in other traits.

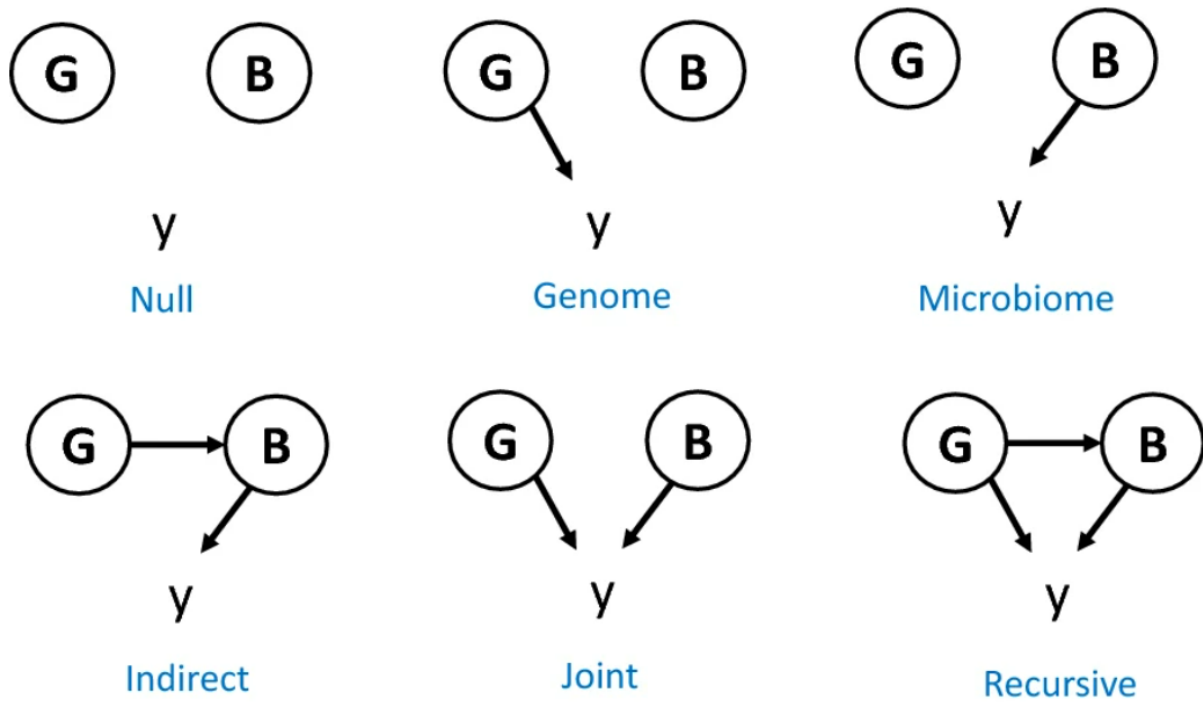
The combination of genomic and microbiomic data often results in more accurate phenotypic predictions than either source alone. Modeling the interaction between the two is proving to be

even more effective. As our knowledge about the functional role of specific genomic loci and microbial features grows, new models may emerge that take advantage of that information.

Most information about the microbiome's relationship with the host comes from dairy cattle, swine, and sheep. There is a great opportunity for exploration in this space for beef cattle researchers. The genetic correlation between microbial features and economically important traits, the best way to model microbial data for genetic/phenotypic prediction, and even something as simple as the microbiability estimates of traits are all scarce in beef cattle. With that knowledge in hand, microbiome data opens whole new realm of possibilities to bolster beef cattle genetic improvement and management.

4 Figures

Figure 1 – Adapted from Pérez-Enciso et al. (2021)



Hypothetical models for how host genetics (G) and microbiome (B) components might affect host phenotype (y).

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