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Manipulation of gut microbiota composition for the genetic selection of the host

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Introduction

The microbiota inhabiting the gastro-intestinal tract. Animal the gut microbiota (GM) has been proposed for its unique potential to describe and interpret complex host phenotypes, reflecting genetic and environmental influences and their interplay. Lastly, the GM has been proposed as the ultimate tool to explain animal resilience, for its nature of representing a dynamic and responsive ecosystem, defined and determined in tight relationship with the animal's physiology. This allows to propose GM as a component of 'animal phenomics', i.e. as a source of information about the animal to be employed in the farming decision-making processes, broadly speaking.
So, to give a broad definition encompassing both ruminants' and monogastrics' physiology, GM could be defined as *the complex ecosystem of symbiont organisms that inhabits the gastro-intestinal tract*. The GM has contributed to a deeper understanding of a wide range of complex traits, including methane emissions (Saborio-Montero *et al*., 2019; Martinez-Alvaro *et al*., 2022; Gonzalez-Recio *et al*., 2023), meat quality (Khanal *et al*., 2020), feeding behavior (He *et al*, 2022), and feed efficiency (Camarinha-Silva *et al*., 2017). Indeed, the GM appears to be reflecting underlying biological processes that connect host genetics with environmental and physiological responses.
This contribution stems from the fact that microbial variation reflects a complex interplay between host genetics, environmental conditions, and internal physiological states such as age, health conditions, and disruptions due to stress. Consequently, the GM is increasingly recognized not only as a biomarker of the host's physiological condition, but also as a promising tool for predicting and selecting complex phenotypes.
Together, these considerations highlight the GM as both an integrative indicator of health and resilience, as well as a complementary source of phenotypic information for genetic improvement, while motivating a broader conceptual framework that views the host and its microbiota as a functional biological unit.

The data-sources. In terms of the sources for GM data, several options have been presented. Multiple 'omics' approaches can be used. These include, but are not limited to, meta-genomics, meta-transcriptomics and meta-bolomics. These offering several opportunities to comprehensively characterize microbial changes across molecular levels (Chakraborty *et al*., 2022; Gheyas *et al*., 2025) Despite advances in the multiple 'omics' technological platforms, most studies in livestock have employed single-omics or at most paired-omics designs. Actually, many studies with an animal breeding focus have relied on 16S sequencing, probably due to the prohibitive cost of shotgun metagenomics sequencing, although some attempts have been taken (Martinez-Alvaro *et al*., 2022).

The holobiont. The genetic components of the host and the gut microbiota can be viewed as a single integrated unit, commonly known as the holobiont, as mentioned in the previous section. The holobiont concept has its roots dating back to the 19th and early 20th century, when it was hypothesized that mitochondria and chloroplasts could have been symbiotic bacteria that are now systematically transmitted through the cytoplasm of the host's cells (Baldauf *et al*., 2003).

Then, the holobiont was defined the holobiont as "a multispecies consortium of cells with many genomes that can contribute to multiple functions throughout the body" (Carrier and Reitzel, 2017).
Biologically, the holo-biont is formed and functions when the conditions of:

1-The presence of abundant and diverse microbiota, and the possibility for the host to acquire different symbionts.
2-The fidelity of transmission of the microbiota from parent to offspring apply.
3-The impact of the symbiotic microbiota on the host fitness,

From a breeding perspective, the three parts can be seen respectively as 1) a factor determining phenotypic variation in GM, 2) a non-zero value of heritability of the GM, 3) a factor determining selection intensity, realized the host fitness due to its GM. From a quantitative genetic perspective, selecting animals with microbiota profiles enriched in favourable taxa could potentially improve the traits of interest, as these animals may be better equipped to cope with environmental and physiological stressors. This hypothesis is supported by the reverse phenomenon having occurred: while selecting for the most efficient animals, a more efficient GM has been selected for (Bergamaschi *et al*., 2020).
All in all, the three conditions for the formation and functioning of the holobiont propose GM as an additional feature to be added to the statistical models used for animal breeding, either as correlated indicator trait or an additional effect in the model. While the statistical difference would be fundamental, in this paper we will skip over this problem to focus on the handling of GM information itself, highlighting pros and cons of each approach.

Materials & Methods

A problem stems from the large amount of information contained in GM data. This manifests in both a statistical problem and a interpretability issue. In the first, the statistical models developed for animal breeding are not suited for handling large amount of microbial information, both as an additional GM-based effect and as indicator traits in selection. As for the second one, the dynamic and compositions nature of the GM could lead to uncompelling proposals of their use in practical breeding.
Here, we provide an overview of the different methods found in literature to use GM information in statistical models oriented at animal breeding. While the overview may not be totally exhaustive, it should provide an idea of the pros and cons of each approach.

Results

Pros and cons of using different methodological approaches to include gut microbial information into statistical models for the genetic selection of the host are reported in table 1.

Discussion

The different approaches are discussed within the table. Currently, there is lack of direct comparison of the different methods for animal breeding purposes. This comparison should be carried out under different scenarios, encompassing biological variability between and within ruminants and monogastrics, in addition to factors such as diet and farming conditions

Conclusions

Different methods are available to include gut microbial information as a source of phenomic information. Their inclusion in statistical models for the genetic selection of the host will likely be developed in the upcoming years. While none of the methods appear to be flawless, animal breeders should be acquire deep knowledge in the ecological definition of gut microbial information before any method is implemented in genetic evaluations.

Table 1. Pros and cons of using different methodological approaches to include gut microbial information into statistical models for the genetic selection of the host.

Pros	Cons
Single feature (ASV or OTU)[1]	
Simple workflow, no additional steps after sequencing and bioinformatic assignment. Relative abundance of single taxa may meet the assumptions of the infinitesimal model.	Very sensitive to the identification and quantification with 16S sequencing. Maybe hard to improve as a quantitative trait. Error (random or systematic) in sequencing and bioinformatics steps could lead to inefficiencies in selection.
Ratio between taxa (e.g. Firmicutes/Bacteroidetes) and Alpha-diversity metrics [2]	
A single trait might picture a broad dynamic happening between interacting taxa. Some ratios show strong association with economically relevant traits. Could allow to macroscopically shift microbial composition towards a more favourable one, without compromising its functioning at the microscopic level. In general, higher diversity means higher 'health' of an ecosystem. In animal breeding this could easily related to higher genetic variability, making it easy to interpret.	Ratio traits are difficult to interpret biologically and lead to uncomfortable interpretation in genetic evaluations. Indices picture the diversity of the ecosystem, not necessarily its (favourable) composition. There is no proven and accepted rationale in considering a diversity index as a trait: two individuals with high diversity might have totall different composition, which would strongly compromise genetic progress. We could seriously oversimplify the ecological processes undergoing the interaction between the microbiota and the host.
Principal components or coordinates[3]	
Strong dimensionality reduction. Very convenient to be used for multiple-trait models. The machinery for the inclusion of factors or components into multiple-trait models has been proven to be effective.	Statistical reduction of dimensionality inevitably leads to loss of biological information. We could lose information in the attempt to remove its redundant component. Unsupervised methods for dimensionality reduction could need some constant retraining, which could be laborious.
Enterotypes and guilds (functional) [4]	
Strong dimensionality reduction. Very convenient for multiple-trait models. Could be closely linked to the metabolomic functioning of the host (if based on functional information). Can provide easy biological interpretability and efficient use in statistical models for animal breeding.	Likely needs to rely on taxonomic or functional information to be sourced externally, leading to unstable determination of clusters. Proof of its effectiveness in selection is yet to be obtained. The different bioinformatic steps could lead to unstable assignment of samples/individuals to clusters, with consequent unreliable definition of phenotypes.
Entero-signatures (taxonomic or functional)[5]	
Strong dimensionality reduction. Very convenient for multiple-trait models. The clustering based on co-occurrence and the continuous definition of the trait for each sample is convenient for its use in statistical models for animal breeding.	Definition of entero-signature groups is unsupervised; dimensionality reduction algorithm could constrain potentiality of the method. The method is data-dependent: adding additional records could alter assignment of the previously existing records to each group.

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