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AN OVERVIEW ABOUT GUT MICROBIOTA OF PIGS IN FEED EFFICIENCY

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Abstract

The aim of this study was to investigate the relationship between the gut microbiota and feed efficiency, which is an important parameter in pig production, with economic and environmental impact. The gut microbiota has a fundamental responsibility in nutrient digestibility and influences feed efficacy and metabolism. It provides several functions: supply of enzymes which improve the feed value, metabolism of feed to produce nutrients and synthesis of vitamins useful to the animal body. The animals develop a gut microbiota over time and space. For pigs, the scientists use 16S rRNA amplicon metagenomic sequencing for identify bacteria species and 18S rRNA for fungi. The gut microbiota is an important component of the growth variability in all living organisms, and microbiota knowledge could change the actions to obtain a sustainable and efficient lean meat production.

Key words*: FE, feed, meat, metabolism, pig.*

INTRODUCTION

The attention paid to the intestinal flora in humans, but also in pigs, significantly increased. In the last 15 years more than 20,000 papers have been published on this topic. Due to the diversification of the methodology for identifying microbial species, scientists have discovered new methods to treat various pathologies of the digestive tract, respiratory tract, and ways to manipulate the microbiota, the communities of microorganisms in the gut, for nutritional purposes (McCormack et al., 2017).

The complexity of the microscopic world in the intestine with its involvement in the metabolic and immunological functions of the macroorganism created the title of "new organ" (Ramayo-Caldas et al., 2016).

Volatile fatty acids resulting from microbial metabolism have been shown to interact with intestinal mucosal cells by increasing the absorption of nutrients and thus increasing feed efficiency (FE) (Shirkey et al., 2006; Willing & Van Kessel, 2010).

After birth the animal body is populated with an enormous variety of bacteria, protozoa, archaea, fungi and viruses, whose number varies between 10 and 100 times the total number of cells in the body. The proportion between species of microorganisms changes with increasing and changing diet, for example: weaning, but also by genetic factors such as the host (Thursby & Juge, 2017).

With the improvement of genome segmentation technology, tens of thousands of entities with different functions that regulate the homeostasis of the organism as a whole have been identified and can be considered as an additional organ. To better characterize the phenomenon, two different terms have been introduced: the microbiome, which defines the collection of genomes from all microorganisms in the environment, and the microbiota, which identifies specific microorganisms that are found in a specific environment (Bergamaschi et al., 2020).

MATERIALS AND METHODS

Metagenome is based on diversity and functional prediction. The methodology is realized on 16S ribosomal RNA gene sequencing. 16S/18S/ITS amplicon metagenomic sequencing is frequently used to identify and differentiate microbial species. Short (<500 bp) hypervariable regions of conserved genes or intergenic regions, such as 16S of bacteria and archaea or 18S/ITS of fungi, are amplified by PCR and analyzed

using next generation sequencing (NGS) technology (Novogene - High Quality Gene Sequencing).

The resulting sequences are compared against microbial databases. Applications range from identifying a single species in pure culture and characterizing the microbiota of animals or plants, to comparing species diversity and population structure from various environmental sources or geographic regions.

Metagenomic biomarker is a test of biological consistency and effect size estimation. This addresses the challenge of finding organisms, genes, or pathways that consistently explain the differences between two or more microbial communities, which is a central problem to the study of metagenomics. The method was validated on several microbiomes and a convenient online interface for the method is provided at http://huttenhower.sph.harvard.edu/lefse/ (The Huttenhower Lab, 2021).

Such samples can be analyzed by high-speed DNA sequencing methods, known as metagenomics, metabarcoding, and single-species detection, for rapid monitoring and measurement of biodiversity. To better differentiate between organisms in a sample, DNA metabarcoding in which the sample is analyzed is used and previously studied DNA libraries, such as Basic Local Alignment Search Tool, are used to determine which organisms are present.

The resulting biological observation matrix files were normalized according to known/predicted 16S rRNA gene copy numbers, and the metagenomes were predicted using precalculated Kyoto encyclopedia of genes and genomes (KEGG) orthologs.

Profiling phylogenetic marker genes, such as the 16S rRNA gene, is a key tool for studies of microbial communities but does not provide direct evidence of a community's functional capabilities. It was used the PICRUSt (phylogenetic investigation of communities by reconstruction of unobserved states), a computational approach to predict the functional composition of a metagenome using marker gene data and a database of reference genomes. PICRUSt uses an extended ancestral-state reconstruction algorithm to predict which gene families are present and then combines gene families to estimate the composite metagenome. Using 16S information, PICRUSt recaptures key findings from the Human Microbiome Project and accurately predicts the abundance of gene families in host-associated and environmental communities, with quantifiable uncertainty. The results demonstrate that phylogeny and function are sufficiently linked that this 'predictive metagenomic' approach should provide useful insights into the thousands of uncultivated microbial communities for which only marker gene surveys are currently available (Langille et al., 2013).

PCR based on deep pyrosequencing of the 454 platform has revealed extensive microbial diversity that was previously undetected with culture-dependent methods.

DNA sequencing allows the use of data to identify and classify the Bacteria and Archaea microorganisms. EzBioCloud is an integrated database, where the taxonomic hierarchy of Bacteria and Archaea is located. At the genome level are important information's, which contributes to the species taxonomy description. Taxonomically significant information about species can be extracted and statistically compiled using species multiple genomes (Yoon et al., 2017).

The codes for metagenomic analyses are publicly available at https://github.com/strowig-lab/PIBAC, referenced under https://doi.org/10.5281/zenodo.4075065.

The latest bacterial collection from pig intestine can be verified in the article published in Nature Communication by Wylensek et al. (2020).

For the microbial functional prediction, a more accurate evaluation of the biological values is made by correlating the microbiotic profile with the feed efficiency (FE). A series of tests are used such as: salivary cortisol, serum haematological and biochemical tests, immunological tests, serum haptoglobin, lipopolysaccharides in cecal digestion, microbiota profile, concentrations of volatile fatty acids in faeces and digestion (McCormack et al., 2019b).

RESULTS AND DISCUSSIONS

In the past, the relationship between the host and the intestinal microbiota was known as a commensalism or a parasitism; however, recent

researches revealed their relationship as mutualism. It is important to choose the proper feed additives for the growth stage, and therefore, an understanding of the alteration of the intestinal microbiota with the growth of pigs is required (Ramayo-Caldas et al., 2016).

The practical application for correcting intestinal microbial imbalances, but especially for improving the nutritional performance of feed, is the fecal microbiota transplant (FMT). The use of (FMT) showed that growth performance increased significantly without changing the overall microbiome of the subjects (Wang et al., 2019).

From genera co-occurrence network analysis, we revealed several relationships within the swine intestinal microbiota at various growth stages. Overall, a positive correlation was observed between the genera within the same phylum, while a negative correlation was observed between the genera belonging to the different phylum, with some exceptions. The enzymatic equipment of the microbiota contributes to the destructuring of the feed producing metabolites with direct influence on the physiology of the host organism.

The resulting biological observation matrix files was normalized according to known/predicted 16S rRNA gene copy numbers, and the metagenomes were predicted using precalculated KEGG orthologs.

In conclusion, the FE-associated bacterial taxa consistently found across rearing environments may have a role to play in improving FE in pigs, mainly because of their importance in relation to carbohydrate metabolism. In addition, methanogenic members of the Archaea (*Methanobrevibacter*) are also likely to shape FE in pigs. In the future, these FE-associated taxa could potentially be used as probiotics or targeted by dietary means as a strategy for improving FE in pigs. Alternatively, they could be exploited as potential predictive biomarkers for porcine FE (McCormack et al., 2019b).

Xiao et al., in 2016, identified 7.7 million non-redundant genes, representing 719 metagenomic species, by deep sequencing the fecal DNA metagenome from 287 pigs. The study showed that the sex, age and genetics of the host influence the intestinal microbiome of the pig. Analysis of the prevalence of antibiotic resistance genes has demonstrated the effect of eliminating antibiotics from animal diets and therefore reducing the risk of spreading antibiotic resistance associated with agricultural systems. *Firmicutes, Bacteroidetes, Actinobacteria, Spirochaetes* and *Proteobacteria* were the five dominant threads found in the specimens. The microbial diversity of females was significantly higher compared to males; castration increased the intestinal microbial diversity of males. The functional prediction showed that the metabolism of cofactors and vitamins were also rich in the female group; fecal microorganisms of castrated males influenced membrane transport in enterocytes. The genera *Prevotella* and *Ruminococcus* were consistent with the two enterotype groups identified in the pig microbiota (Xiao et al., 2016).

Recently, studies have demonstrated associations of microbial profiles with nutrition and productivity parameters. Notably, the gut microbiota metabolizes various food components, providing nutrients to the host in the form of fermentation end-products and other by-products, amino acids, vitamins, and indole derivatives. In the context of swine FE, the gut microbiota plays important roles in nutrient uptake, energy harvest, and carbohydrate metabolism, particularly in processing indigestible polysaccharides (Yang et al., 2017). Recent studies have reported that the composition of the pig gut microbiota are correlated with nutrient digestibility, average daily gain, and body weight. Variation in the gut microbiome has also been associated with life stage. However, to the best of our knowledge, only a few studies have reported the effect of different microbial populations on feeding efficiency of different breeds. Singh et al., in 2014, reported a correlation between gut microbiota diversity and FE, while Tan et al., in 2017, identified differences in the microbiomes of pigs with high and low feed efficiencies.

The genera *Bacteroides*, *Cellulosilyticum*, and *Prevotella,* were more abundant in low FE pigs, and *Oscillibacter* and *Rhodococcus* were found in animals that were more feed efficient. It is expected that host genetics has the potential to meaningfully influence the gut microbiota, and consequently FE, by favouring

or disfavouring microbes that significantly contribute to nutrient digestion and energy harvest. Therefore, the gut microbiome composition could be associated with intestinal morphology and physiology that can impact the production traits such as growth and feed intake (Tan et al., 2017).

Characterizing the relationship between gut microbial composition and FE revealed a positive association between four genera (*Lactobacillus*, *Blautia*, *Dorea*, and *Eubacterium*) and FE. Moreover, previous studies in swine demonstrated that an increase in the production of short-chain fatty acids could improve the absorptive capacity of the intestine, promoting the growth of beneficial bacteria, thereby increasing FE (Yang et al., 2017; Bergamaschi et al., 2020).

The functional prediction of the microbiome shows that bacterial community interactions in the gut is very complex and the overall functions of the microbiome as a community outweighs the contribution of a single member of that community (Umu et al., 2020).

Microbial diversity varied by geographic location and intestinal sampling site but not by Residual Feed Intake (RFI) rank, except in one geografical location, where more-feed-efficient pigs had greater ileal and cecal diversity. Although none of the 188 RFI-associated taxonomic differences found were common to all locations/batches, *Lentisphaerae*, *Ruminococcaceae*, F16, *Mucispirillum*, *Methanobrevibacter*, and two uncultured genera were more abundant within the fecal or cecal microbiota of low-RFI pigs in two geographic locations and/or in both other geographic location batches. These are major contributors to carbohydrate metabolism, which was reflected in functional predictions. Fecal volatile fatty acids and salivary cortisol were the only physiological parameters that differed between RFI ranks (McCormack et al., 2017).

The gut microbiota is an essential requirement for host health and it performs many functions. These include: driving intestinal development, strengthening intestinal barrier function and controlling epithelial cell proliferation; the provision of enzymes which increase the value of food; metabolism of non-digestible foods to produce nutrients useful to the host; and synthesis of vitamins which cannot be consumed or generated by the host (Lewis, 2013; Pajarillo et al., 2014).

The major ingredients of formula diet provided to the experimental pigs in this study included fibber-enriched corn and high-protein soybean. Therefore, we hypothesized that gut microbiome of the high feed-efficiency pigs might have a greater ability to utilize the dietary indigestible cellulose. The Short Chain Fatty Acids (SCFAs) produced by fermenting dietary polysaccharide are the preferred energy source rather than glucose and lactose for colonic mucosa (Pryde et al., 2002). Moreover, SCFAs could reduce intestinal inflammation, which improves the absorptive capacity of intestine, and increases porcine FE. Interestingly, *L. casei* was also identified to enrich in the high feed-efficiency pigs. As a probiotic, *Lactobacillus* can promote intestinal development and metabolism. The study in chicken showed that *Lactobacillus johnsonii BS15* promotes growth performance and lowers fat deposition (Wang et al., 2017).

These results suggested that the gut microbiome of pigs with the high FE have a greater ability to utilize the dietary polysaccharides and protein. It was inferred that gut microbiota might improve porcine FE through promoting intestinal health by the SCFAs produced by fermenting dietary polysaccharides. However, the functional capacities of gut microbiome inducing fatness might reduce porcine FE. These results provide important insights into how gut microbiome influences porcine FE, and gave the basic knowledge for improving porcine FE through modulating the gut microbiota in pig industry (Yang et al., 2017).

CONCLUSIONS

Studies have shown the direct involvement of the intestinal microbiome in the physiological processes of enterocyte levels by altering membrane transport mechanisms, carbohydrate metabolism and glycocalyx formation.

Intestinal microbiom ecosystems influence the growth rate of pigs. In the future, their handling may lead to an increase in feed efficiency, with implications for production costs and environmental protection

REFERENCES

Bergamaschi, M., Tiezzi, F., Howard, J., Huang, Y.J., Gray, K.A., Schillebeeckx, C., McNulty, N.P., & Maltecca, C. (2020). Gut microbiome composition differences among breeds impact feed efficiency in swine. *Microbiome*, *8*(1):110. doi: 10.1186/s40168-020-00888-9. PMID: 32698902; PMCID: PMC7376719

Burger-van Paassen, N., Vincent, A., Puiman, P.J., van der Sluis, M., Bouma, J., Boehm, G., van Goudoever, J.B., van Seuningen, I., & Renes, I.B. (2009). The regulation of intestinal mucin MUC2 expression by short-chain fatty acids: implications for epithelial protection. *Biochem. J.*, *420*(2), 211-219. doi: 10.1042/BJ20082222.

GitHub (2020). In-depth cultivation of the pig gut microbiome towards novel bacterial diversity and tailored functional studies – PIBAC. https://github.com/strowig-lab/PIBAC. doi: 10.5281/zenodo.4075065

Langille, M.G.I., Zaneveld, J., Caporaso, G., McDonald, D., Knights, D., Reyes, J.A., Clemente, J.C., Burkepile, D.E., Thurber, R.L.V., Knight, R., Beiko, R.G., & Huttenhower, C. (2013). Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. *Nature Biotechnology*, *31*(9), 814-821. doi: 10.1038/nbt.2676.

Lewis, M. (2013). A pig's microbiota – a result of many factors. *Pig Progress*, *29*(8), 24-26. Retrieved October 10, 2020, from https://www.pigprogress.net/Piglets/Articles/2013/11/A-pigs-microbiota--a-result-of-many-factors-1378759W/

Maltecca, C., Bergamaschi, M., & Tiezzi, F. (2020). The interaction between microbiome and pig efficiency: a review. *J. Anim. Breed Genet.*, *137*(1), 4-13, doi: 10.1111/jbg.12443.

McCormack, U.M., Curião, T., Buzoianu S.G., Prieto, M.L., Ryan, T., Varley P., Crispie, F., Magowan, E., Metzler-Zebeli, B.U., Berry, D., O'Sullivan, O., Cotter, P.D., Gardiner G.E., & Lawlor, P.G. (2017). Exploring a possible link between the intestinal microbiota and feed efficiency in pigs. *Appl. Environ. Microbiol.*, *83*(15), e00380-17. doi 10.1128/AEM.00380-17.

McCormack, U.M., Curião, T., Metzler-Zebeli, B., Magowan, E., Berry, D., Reyer, H., Prieto, M.L., Buzoianu, S., Harrison, M, Rebeiz, N., Crispie, F., Cotter, P.D., O'Sullivan, O., Gardiner G.E., & Lawlor P.G. (2019a). Porcine feed efficiency-associated intestinal microbiota and physiological traits: finding consistent cross-locational biomarkers for residual feed intake. *mSystems*, 4. doi: 10.1128/mSystems.00324-18

McCormack, U.M., Curião, T., Metzler-Zebeli, B.U., Wilkinson, T., Reyer, H., Crispie, F., Cotter, P.D., Creevey, C.J., Gardiner, G.E., & Lawlor, P.G. (2019b). Implementation of feed efficiency in pigs through microbial modulation via fecal microbiota transplantation in sows and dietary supplementation of inulin in offspring. *Appl. Environ. Microbiol.*, *85*(22), e01255-19. doi: 10.1128/AEM.01255-19.

Pajarillo, E.A.B., Chae, J.P., Balolong, M.P., Kim, H.B., Seo K.S., & Kang, D.K., (2014). Pyrosequencing-based analysis of fecal microbial communities in three purebred pig lines. *J. Microbiol.*, *52*(8), 646–51. doi: 10.1007/s12275-014-4270-2

Pryde, S.E., Duncan, S.H., Hold, G.L., Stewart, C.S., & Flint, H.J. (2002). The microbiology of butyrate formation in the human colon. *FEMS Microbiol. Lett.*, 217, 133–139. doi: 10.1111/j.1574-6968.2002.tb11467.x

Ramayo-Caldas, Y., Mach, N., Lepage, P., Levenez, F., Denis, C., Lemonnier, G., Leplat, J.J., Billon, Y., Berri, M., Dore, J., Rogel-Gaillard, C., & Estellé, J. (2016). Phylogenetic network analysis applied to pig gut microbiota identifies an ecosystem structure linked with growth traits. *ISME J.*, *10*(12), 2973–2977. doi: 10.1038/ismej.2016.77.

Ríos-Covián, D., Ruas-Madiedo, P., Margolles, A., Gueimonde, M., de Los Reyes-Gavilán, C.G., & Salazar, N. (2016). Intestinal short chain fatty acids and their link with diet and human health. *Front Microbiol*., 7, 185. doi: 10.3389/fmicb.2016.00185.

Shirkey, T.W., Siggers, R.H., Goldade, B.G, Marshall, J.K., Drew, M.D., Laarveld, B., & Van Kessel, A.G. (2006). Effects of commensal bacteria on intestinal morphology and expression of proinflammatory cytokines in the gnotobiotic pig. *Exp. Biol. Med. (Maywood)*, *231*(8), 1333–1345. doi: 10.1177/153537020623100807.

Singh, K.M., Shah, T.M., Reddy, B., Deshpande, S., Rank, D.N., & Joshi, C.G. (2014). Taxonomic and gene-centric metagenomics of the fecal microbiome of low and high feed conversion ratio (FCR) broilers. *J. Appl. Genet.*, *55* (1), 145-154. doi: 10.1007/s13353-013-0179-4.

Tan, Z., Yang, T., Wang, Y., Xing, K., Zhang, F., Zhao, X., Ao, H., Chen, S., Liu, J., & Wang, C. (2017). Metagenomic analysis of cecal microbiome identified microbiota and functional capacities associated with feed efficiency in Landrace finishing pigs. *Front Microbiol.*, *11*(8), 1546. doi: 10.3389/fmicb.2017.01546.

The Huttenhower Lab (2021, February 15). *LEfSe (Linear discriminant balysis Effect Size)*. http://huttenhower.sph.harvard.edu/lefse/.

Thursby, E., & Juge, N. (2017). Introduction to the human gut microbiota. *Biochem. J.*, *474*(11), 1823–1836. doi: 10.1042/BCJ20160510

Umu, Ö.C.O., Mydland, L.T., Øverland, M., McLean Press, C., & Sørum, H. (2020). Rapeseed-based diet modulates the imputed functions of gut microbiome in growing-finishing pigs. *Scientific Reports*, 10, 9372. https://doi.org/10.1038/s41598-020-66364-4.

Wang, X., Tsai, T., Deng, F., Wei, X., Chai, J., Knapp, J., Jason, A., Maxwell, C., Lee, J., Li, Y., & Zhao, J. (2019). Longitudinal investigation of the swine gut microbiome from birth to market reveals stage and growth performance associated bacteria. *Microbiome*, 7, 109. doi: 10.1186/s40168-019-0721-7.

Willing, B.P., & Van Kessel, A.G. (2010). Host pathways for recognition: establishing gastrointestinal microbiota as relevant in animal

health and nutrition. *Livest Sci.*, *133*(1/3), 82–91. doi:10.1016/j.livsci.2010.06.031.

Wu, W., Zhang, L., Xia, B., Tang, S., Lei, L., Xie, J., & Zhang, H. (2020). Bioregional alterations in gut microbiome contribute to the plasma metabolomic changes in pigs fed with inulin. *Microorganisms*, *8*(1), 111. doi: 10.3390/microorganisms8010111.

Wylensek, D., Hitch, T., Riedel, T., Afrizal, A., Kumar, N., Wortmann, E., Liu, T., Devendran, S., Lesker, T.R., Hernàndez, S.B., Heine, V., Buhl, E.M., D'Agostino, P.M., Cumbo F., Fischöder, T., Wyschkon, M., Looft, T., Parrira, V.R., Abt, B., Doden, H.L., Ly, L., Alvez, J.M.P., Reichlin, M., Flisikowski, K., Suarez, l.N., Neumann, A.P., Suen, G., de Wouter, T., Rohn, S., Lagkouvardos, I., Allen-Vercoe, E., Spröer, C., Bunk, B., Taverne-Thiele A.J., Giesbers, M., Wells, J.M., Neuhaus, K., Schnieke, A., Cava, F., Segata, N., Elling, L., Strowig T., Ridlon, J.M., Gulder, T.A.M., Overmenn, J., & Clavel, T. (2020). A collection of bacterial isolates from the pig intestine reveals functional and taxonomic diversity. *Nature Communications*, *11*(1), 6389. doi: 10.1038/s41467-020-19929-w.

Xiao, L, Estellé, J., Kiilerich, P., Ramayo-Caldas, Y., Xia, Z., Feng, Q., Liang, S., Pedersen, A.Ø., Kjeldsen, N.J., Liu, C., Maguin, E, Doré, J., Pons, N., Le Chatelier, E., Prifti, E., Junhua, L., Jia, H., Liu, X., Xu, X., Ehrlich, S.D., Madsen, L., Kristiansen, K., Rogel-Gaillard, C., & Wang, J. (2016). A reference gene catalogue of the pig gut microbiome. *Nature Microbiology*, 1, 16161. doi: 10.1038/nmicrobiol.2016.161.

Yang, H., Huang, X., Fang, S., He, M., Zhao, Y., Wu, Z., Yang, M., Zhang, Z., Chen, C., & Huang L. (2017). Unraveling the fecal microbiota and metagenomic functional capacity associated with feed efficiency in pigs. *Frontiers in Microbiology*, 8, 1555. doi: 10.3389/fmicb.2017.01555.

Yoon, S.H., Ha, S.M., Kwon, S., Lim, J., Kim, Y., Seo, H., & Chun J. (2017). Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and hole-genome assemblies. *Int J Syst Evol Microbiol*., *67*(5), 1613–1617. doi: 10.1099/ijsem.0.001755.