

# The sow microbiome: Current and future perspectives to maximize the productivity in swine herds

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## Summary

The development of new generation sequencing methods and the reduction in the cost per base sequenced over the past few years is drawing the attention of the pig industry to microbiome understanding and modulation. In recent years, there has been an increase in the number of articles published related to microbiome studies in swine. With respect to sows, microbiome studies mainly focused on the gut, with some studies evaluating the reproductive tract and mammary microbiome. However,

studies about urinary microbiome are still lacking. The present literature indicates that the microbiome in the sow's gut can affect the microbiome in other body parts. Moreover, the understanding of the dynamics and interactions among microbial populations within the sow or the herd has led to improvements in animal health and reproductive performance. This review provides new insights related to sow intestinal, urinary, mammary, and reproductive microbiomes and their relationships with reproductive outcomes, diseases, and early colonization in offspring by

gathering the most recent work in this field as well as pinpointing information gaps that require further investigation. This literature review also sheds light on the knowledge regarding the role of microbiomes in the reduction of antimicrobial use.

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## Resumen - El microbioma de la cerda: Perspectivas actuales y futuras para maximizar la productividad en las piaras porcinas

En los últimos años el desarrollo de métodos de secuenciación de nueva generación y la reducción en el costo por base secuenciada está atrayendo la atención de la industria porcina hacia la comprensión y modulación del microbioma. En los últimos años, ha habido un aumento en el número de artículos publicados relacionados con estudios del microbioma en cerdos. Con respecto a las cerdas, los estudios del microbioma se centraron principalmente en el intestino, con

algunos estudios que evaluaron el tracto reproductivo y el microbioma mamario. Sin embargo, todavía faltan estudios sobre el microbioma urinario. La literatura actual indica que el microbioma en el intestino de la cerda puede afectar el microbioma en otras partes del cuerpo. Además, la comprensión de la dinámica y las interacciones entre las poblaciones microbianas de la cerda o de la piara han llevado a mejoras en la salud animal y el rendimiento reproductivo. Esta revisión de los trabajos más recientes en esta área proporciona nueva información relacionada con los microbiomas intestinales, urinarios, mamarios, y

reproductivos de las cerdas, su relación con los resultados reproductivos, las enfermedades, y la colonización temprana de su progenie e indica también la falta de información que requiere mayor investigación. Esta revisión de la literatura también se expone el conocimiento del rol de los microbiomas en la reducción del uso de antimicrobianos.

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## Résumé - Le microbiome de la truie: Perspectives actuelles et futures pour maximiser la productivité des troupeaux porcins

Le développement de méthodes de séquençage de nouvelle génération et la réduction du coût par base séquencée ces dernières années attirent l'attention de la filière porcine sur la compréhension et la modulation du microbiome. Au cours des dernières années, il y a eu une augmentation du nombre d'articles publiés liés aux études sur le microbiome chez le porc. En ce qui concerne les truies, les études sur le microbiome

se sont principalement concentrées sur l'intestin, certaines études évaluant l'appareil reproducteur et le microbiome mammaire. Cependant, les études sur le microbiome urinaire font encore défaut. La littérature actuelle indique que le microbiome dans l'intestin de la truie peut affecter le microbiome dans d'autres parties du corps. De plus, la compréhension de la dynamique et des interactions entre les populations microbiennes au sein de la truie ou du troupeau a permis d'améliorer la santé et les performances de reproduction des animaux. Cette revue fournit de nouvelles

informations sur les microbiomes intestinaux, urinaires, mammaires, et reproducteurs des truies et leurs relations avec les résultats de la reproduction, les maladies, et la colonisation précoce de la progéniture en rassemblant les travaux les plus récents dans ce domaine et en identifiant les lacunes en matière d'informations qui nécessitent une recherche plus approfondie. Cette revue de la littérature met également en lumière les connaissances concernant le rôle des microbiomes dans la réduction de l'utilisation des antimicrobiens.

Productivity of the sow herd is traditionally measured by the number of pigs weaned<sup>1</sup> or kilograms of piglets weaned per sow per year.<sup>2</sup> Longevity is another factor that can impact herd productivity and is directly affected by disease.<sup>3</sup> Antimicrobials are used in all production phases of pig production; and with respect to the sow, they are more frequently used during the lactation phase.<sup>4</sup> Reproductive failures and diseases frequently associated with polymicrobial organisms are traditionally controlled with use of in-feed, broad-spectrum antimicrobials.<sup>5,6</sup> It is estimated that a sow will be treated with at least one active antimicrobial ingredient for an average 3.2 days/year,<sup>7</sup> however this is often underestimated in treatment records.<sup>8</sup> The category of antibiotics used in sows varies greatly between herds, but it was reported that 26% of all herds use antibiotics to treat sows.<sup>9</sup> Rosengren et al<sup>10</sup> reported an incidence of 7.84 sows treated with antibiotics per 1000 sows/day, while Sjölund et al<sup>11</sup> reported an incidence of 42 sows treated with antibiotics per 1000 sows/day. In some herds, all sows were routinely injected with an antimicrobial agent after farrowing.<sup>10</sup> The majority of antimicrobials used in swine herds are classified as critically important or highly important by the World Health Organization.<sup>12</sup> Rosengren et al<sup>10</sup> reported that some herds routinely use ceftiofur for treating sows. The use of third-generation cephalosporins has increased since 2001 and an increase in bacterial isolates from healthy swine showing extended-spectrum, beta-lactamases was observed in the same period.<sup>13</sup> Ceftiofur is restricted to use in animals but is similar to ceftriaxone, which is widely used in human medicine. Therefore, ceftiofur should not be used as a first-choice antimicrobial for sows.<sup>12</sup> The use

of antimicrobials in animal production is a public health matter, as it engenders selection pressure for resistance to antimicrobials. Of all swine, sows are the pigs least treated with antimicrobials.<sup>9,11</sup> Attention should be paid to antimicrobial administration to sows as they can act as a reservoir for transfer of resistant bacteria to their offspring.<sup>4</sup> Due to recent concerns about antimicrobial resistance and the subsequent restrictions on the use of antimicrobials in animal production, researchers are looking for new alternatives to prevent and treat disease. One possible alternative relies on unveiling the mechanisms by which the microbiome interacts with the host and its relationship with health and productivity.<sup>14-16</sup>

The microbiome is defined as a characteristic microbial community occupying a well-defined habitat which has distinct physio-chemical properties and includes the whole spectrum of molecules produced by the microorganisms, their structural elements, metabolites, and molecules produced by the host and are influenced by the surrounding environmental conditions. The microbiome is prone to change in time and scale and is essential for multicellular organism health.<sup>17,18</sup>

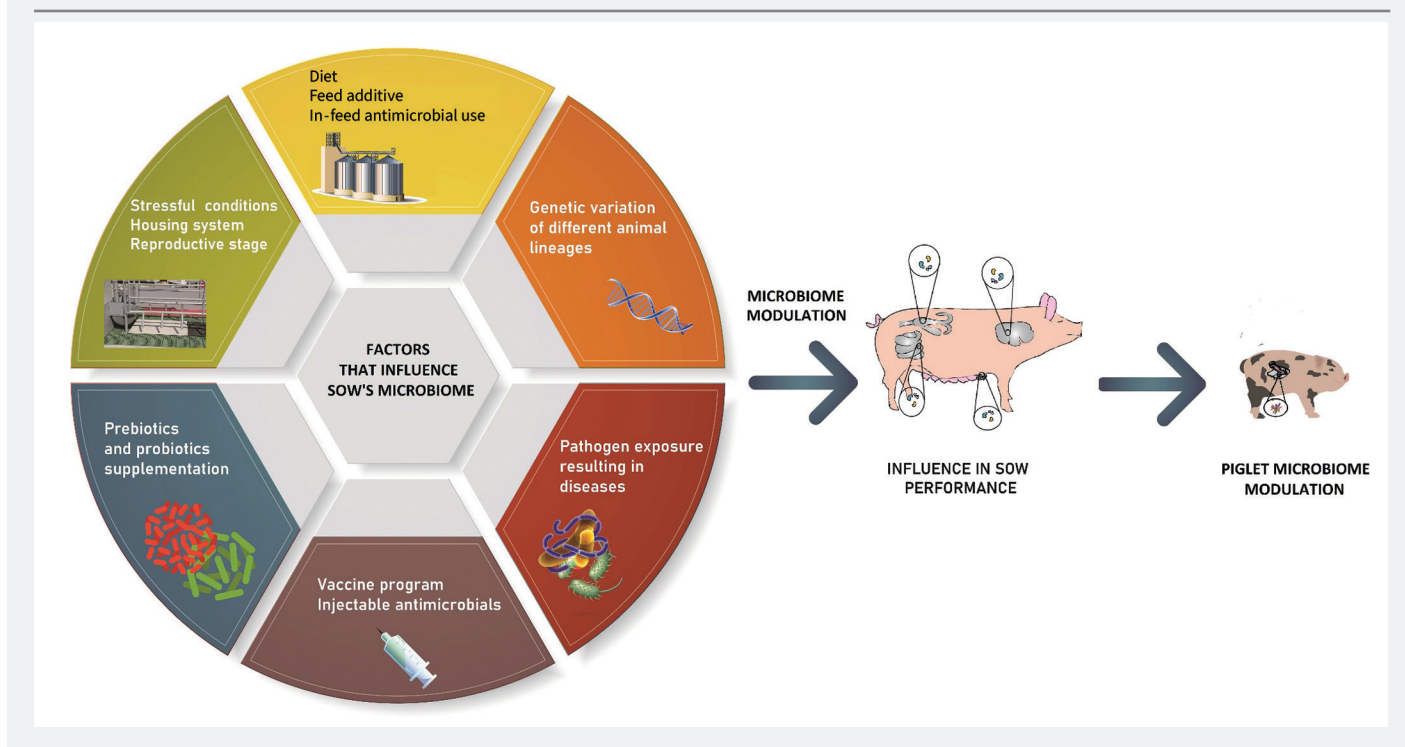
Studies associating the microbiome with disease have been carried out in various species, including humans.<sup>16,19-23</sup> Alterations in vaginal and intestinal microbiomes can reduce urinary tract infections and gut infections in humans.<sup>19,24,25</sup> This new knowledge opens possibilities for new studies to provide a better understanding about microbiome relationships with diseases and reproductive performance. In sows, several factors may alter the microbiome composition. It was reported that antimicrobials

used,<sup>26</sup> reproductive stage,<sup>27</sup> genetic line,<sup>28</sup> feed additives, probiotic and prebiotic supplementation,<sup>29</sup> pathogen exposure, vaccines to prevent disease,<sup>23</sup> and stressful conditions<sup>30</sup> can affect the microbiome. Some of these factors are being studied to increase sow productivity by microbiome modulation<sup>14,15,28</sup> alongside studies investigating the possibility of modulating the offspring microbiome through sow microbiome modulation.<sup>29,31,32</sup> These factors are presented in Figure 1.

In pigs, microbiome modulation can prevent disease and reduce the use of antimicrobials.<sup>33</sup> Pathogen exposures can cause dysbiosis,<sup>23</sup> which can result in an unstable microbiome and increase susceptibility to diseases caused by opportunistic organisms.<sup>34</sup> Both factors contribute to development of disease in sows and impair productivity. Development of a stable microbiome by administration of *Lactobacillus* to newborn piglets has been shown to reduce diarrhea and improve weaning weight.<sup>35,36</sup> Similarly, probiotic supplementation to weaned piglets had a positive effect on average daily gain and reduced diarrhea<sup>37-39</sup> and *Salmonella* shedding.<sup>37</sup> Other studies in swine indicate interaction between the microbiome and other areas of the body. It was observed that *Enterococcus faecalis* EC-12 increased the response of *ex vivo* tissue to immunostimulants such as porcine reproductive and respiratory syndrome virus (PRRSV) modified live virus vaccine.<sup>40</sup> A fecal microbiota transplant (FMT) had beneficial effects in pigs challenged against *Mycoplasma hyopneumoniae*, reducing gross lung pathology.<sup>41</sup>

In sows, there is evidence that changes in the local microbiome (eg, intestinal and vaginal microbiome) may have led to effects in different systems and,

**Figure 1:** Factors that may influence the sow reproductive, urinary, and digestive tracts, colostrum, and milk microbiomes and, consequently, sow performance and the microbiome of their offspring.



consequently, several biomarkers for productivity and optimal health were found.<sup>14,15,30</sup> It was observed that symbiotic supplementation in sows improved their litter performance.<sup>42</sup> It is possible to modulate the sow's microbiome through microbiome transplantation, altering endometrial glands, circulating hormones, and improving reproductive efficiency.<sup>28,43</sup> Research to date has focused mainly on piglet microbiomes, so there is a lack of information regarding the use of probiotics to prevent vaginal discharge, cystitis, mastitis, and diseases that have a great economic impact in sow herds.

For decades, microbiology research has focused on culture methods or detection of individual microbial species or polycultures that may not represent the full bacterial population and diversity since most microorganisms could not be grown by traditional culture methods.<sup>44</sup> The seminal work of Woese and Fox<sup>45</sup> in the 1970s using ribosomal RNA (rRNA) as a bacterial evolutionary marker, mainly with the 16S rRNA gene, revolutionized microbiology research. This and the development of new generation sequencing (NGS) methods have made it possible to characterize the bacterial community in all its richness, diversity, and relative abundance, even in tissues believed to be sterile.<sup>46</sup> Recently, technological

advances have allowed a drastic reduction in sequencing costs, mainly due to the emergence of commercial high-throughput sequencing platforms,<sup>47</sup> and research involving the assessment of the microbiome in swine has gained importance.

Despite the increase of microbiome analysis research, there is a lack of studies correlating the microbiome with its impact on sow productivity. Furthermore, studies that perform organism-based metabolic analysis, identify microbe-microbe interactions, and identify microbe-host interactions are even more scarce. The microbiome is complex, and studies focused on system-based approaches would probably provide more valuable information.<sup>48,49</sup> Thus, this review aims to compile information related to modifications or alterations in the microbiome to improve reproductive performance, as well as to point out topics that require further investigation.

### The reproductive tract microbiome

The number of studies analyzing the vaginal microbiome of sows has increased, especially in the last four years.<sup>14,15,50-54</sup> Studies have focused on identifying

possible biomarkers related to increased productivity,<sup>14</sup> infectious diseases in target sites, ie, endometritis,<sup>16</sup> and immune responses against systemic diseases, such as PRRS. The vaginal microbiome was also studied to identify possible biomarkers for diseases that have an ill-defined biological factor, such as prolapses.<sup>51</sup>

Endometritis has a major impact on the reproductive efficiency of sows<sup>55-58</sup> and its main cause is bacterial infection.<sup>59,60</sup> Common clinical manifestations include purulent vulvar secretion, reproductive failure, abortion, anestrus, reduced farrowing rates, inappetence, and poor body condition which often leads to sow culling.<sup>60,61</sup> This condition could also predispose the sow to other diseases such as postpartum dysgalactia syndrome (PDS) and cystitis.<sup>62,63</sup> Vaginal discharge is the reported reason for 20.5% of culled sows, and endometritis was the most common postmortem lesion (14.5%) in sows culled due to anestrus and repeated breeding.<sup>61</sup>

The application of culture methods associated with biomolecular techniques, notably polymerase chain reaction (PCR), has identified several organisms in purulent vaginal discharge, such as *Escherichia coli*, *Staphylococcus*, *Streptococcus*, *Trueperella pyogenes*,<sup>60,63</sup> *Arcobacter*,<sup>64</sup>

*Chlamydia*,<sup>65</sup> *Proteus*, *Pseudomonas*, and *Corynebacterium*.<sup>63,66</sup> The most common organism found was *E coli*, which was isolated in more than 30% of endometritis cases.<sup>60</sup> Despite the great potential of extraintestinal pathogenic *E coli* to cause metritis, it can also be part of the vaginal microbiome in samples from healthy sows.<sup>16,52-54</sup> The NGS-based studies have corroborated the importance of some of these organisms previously identified by traditional methods, such as *E coli*, *Staphylococcus*, and *Streptococcus*.<sup>16,52</sup> However, NGS metagenomic techniques allow the identification of microbes at a whole community level, in addition to allowing the comparison of relative abundances of each microbe type. This allows for greater resolution to identify organisms which are difficult to identify with traditional methods and may be important in dysbiosis such as low-abundance or fastidious microbes (eg, *Bacteroides*, *Clostridium*, and *Fusobacterium*) recently identified in metagenomic approaches as important pathogenic causes of endometritis.<sup>16,52,53</sup>

Previous studies demonstrated that the vaginal microbiome may act as biological barrier by secreting antimicrobial components such as lactic acid, bacteriocin, and hydrogen peroxide to maintain the health of the reproductive tract.<sup>67,68</sup> Therefore, a sow's vaginal microbiome is complex and even potentially pathogenic bacteria can be part of the community, suggesting that urogenital diseases may arise from dysbiosis.

Wang et al<sup>16</sup> analyzed sow vaginal samples classified as either affected or not affected by endometritis. The Firmicutes phylum was the most abundant (40%-60%) in the vaginal microbiome followed by Proteobacteria (20%-32%) and Bacteroidetes (9%-13%). However, the Firmicutes phylum had the greatest relative abundance in healthy sows, while Proteobacteria and Bacteroidetes were more abundant in samples of sows affected by endometritis. At the genus level, Wang et al<sup>16</sup> found that *Bacillus* and *Paenibacillus* were relatively more abundant in the healthy sows, while *Escherichia-Shigella* and *Bacteroides* were relatively more abundant in sows affected by endometritis. Wang et al<sup>16</sup> observed that one sow with endometritis had a great abundance of *Staphylococcus* during the metagenomic analysis, although the microbial species within the *Staphylococcus* genus was not classified. Experimental inoculation with *Staphylococcus hyicus* caused endometritis in sows in a previous study, as did *E coli*.<sup>59</sup>

Similarly to Wang et al<sup>16</sup>, Zhang et al<sup>52</sup> found that sows with endometritis had a higher relative abundance of *Porphyromonas*, *Clostridium sensu stricto 1*, *Streptococcus*, *Fusobacterium*, *Actinobacillus*, and *Bacteroides* in the birth canal. *Escherichia-Shigella* and *Bacteroides* were higher in the intestines of sows suffering from endometritis, suggesting a link between the onset of endometritis and the increase of these organisms in intestinal microbiota. Xu et al<sup>53</sup> also found the phyla Proteobacteria, Firmicutes, and Bacteroidetes among the most abundant in sow vaginal samples; at the genus level, the most abundant were *Escherichia*, *Streptococcus*, *Enterococcus*, *Bacillus*, *Clostridium sensu stricto 1*, *Staphylococcus*, *Acinetobacter*, *Lactobacillus*, and *Proteus*. Although *Escherichia-Shigella*, *Clostridium sensu stricto 1*, and *Streptococcus* relative abundance were related to endometritis in the other studies,<sup>16,52</sup> no sow had endometritis in the Xu et al<sup>53</sup> study. However, the small number of females evaluated in these two studies (n = 8) precludes stronger conclusions.

Furthermore, Xu et al<sup>53</sup> showed that the addition of lysozyme, an antimicrobial enzyme that occurs naturally in the mucosal barrier of mammals, to the diet of sows affected the vaginal bacterial community by decreasing the relative abundance of *Escherichia-Shigella* and increasing *Lactobacillus*. Members of the Lactobacillaceae family are most abundant in the birth canal of healthy women and are considered protective against infection by other organisms and probiotic candidates.<sup>69</sup> The metagenomic studies related to the vaginal microbiome did not observe a higher prevalence of *Lactobacillus* in healthy sows<sup>16,52</sup> and that even healthy sows carried a higher prevalence of potential pathogenic or opportunistic organisms.<sup>16,52</sup> These results indicate that the sow vaginal microbiome is more complex than what is observed in humans, which contributes to the difficulty of describing a core vaginal microbiome in sows since even discrete changes can impair sow health. Therefore, these authors suggested lysozyme as a candidate for the maintenance of a beneficial vaginal microbiome and consequently reduce the necessity of antimicrobial use to prevent or treat vaginal discharge in the sow herd. Further studies should elucidate the ability of lysozyme to modulate the sow vaginal microbiome for only beneficial microbes.

Sanglard et al<sup>14</sup> evaluated the vaginal microbiome of sows with low and high reproductive performance after PRRSV

vaccination. Sows with low reproductive performance had a higher abundance of noxious bacteria such as *Phascolarctobacterium*, *Filifactor*, *Treponema*, and *Bacteroides* compared to sows with high reproductive performance. *Phascolarctobacterium* was negatively correlated with litter weight at day 21 of lactation<sup>27</sup> and *Filifactor* has been associated with metritis in dairy cows.<sup>70</sup> In addition, discriminant linear analysis using the specific genera *Campylobacter*, *Bacteroides*, *Porphyromonas*, unclassified Lachnospiraceae, *Prevotella*, and *Phascolarctobacterium* was able to differentiate animals with high and low farrowing performance, indicating that these could serve as potential biomarkers.<sup>14</sup> Understanding the vaginal microbiome and potential biomarkers of high reproductive performance may guide improvements in genetic selection at an early age, even prior to breeding. Sanglard et al<sup>14</sup> verified that this method is minimally invasive and can be performed at early ages, such as 4 and 52 days after PRRSV vaccination (132 ± 12 days of age).

Another study<sup>50</sup> investigated the relationship between the vaginal microbiome and sow genetics and the impact on immune response and farrowing traits in commercial gilts. It was found that the genotype was able to explain up to 33% of the immune response variation to vaccination and 14% of the total microbial variation of the vaginal microbiome. The results indicated that the microbiome can be modulated by genetic selection for beneficial microbes, which may indirectly improve reproductive performance, and the possibility to genetically select sows for a better immune response.<sup>50</sup>

The diversity of the vaginal microbiome has been discussed in recent years. Laguardia-Nascimento et al<sup>71</sup> found great variability in the vaginal microbiome of cows, which contradicted previous studies that used culture methods. Sanglard et al<sup>14</sup> found that the microbiome of sows with low reproductive performance had greater vaginal microbial diversity compared to sows with high reproductive performance.

Another factor that contributes to impaired herd productivity is pelvic organ prolapse. Prolapses are more prevalent during late gestation and early lactation and contributes to approximately 21% of sow mortalities annually.<sup>51,72</sup> Sow mortality during the peripartum period is economically critical because

it increases nonproductive days and impairs neonatal nutrition. Despite the great impact of prolapses, prevention is in part neglected due to an ill-defined biological factor. Kiefer et al<sup>51</sup> observed that alpha diversity revealed no significant differences between samples for species richness, community evenness, and diversity. But when analyzed with linear discriminant analysis, there was abundant differences in 89 total operational taxonomic units between sows with high and low prolapse risk. A higher abundance of *Prevotellaceae*, *Treponema*, and *Streptococcus dysgalactiae* was observed in high prolapse risk sows. However, principal coordinate analyses revealed no distinct clustering of sows with high or low prolapse risk and the putative markers identified in this work will require determination of causality.<sup>49</sup> While the Sanglard et al,<sup>14,50</sup> Wang et al,<sup>16</sup> Kiefer et al,<sup>51</sup> Zhang et al,<sup>52</sup> and Xu et al<sup>53</sup> studies were not designed to describe a core vaginal microbiome community associated with better reproductive outcomes in sows, they do show that some changes in bacterial composition may influence a sow's disease response and reproductive performance. Further studies focusing on system-based approaches are required to understand the role of the microbiome in reproductive performance.

## The urinary tract microbiome

Urinary tract infections (UTIs) have great prevalence in swine herds and cause economic losses due to reproductive failures, increased sow culling, and mortality.<sup>73,74</sup> It was reported that more than 90% of sows with some reproductive disorder also were diagnosed with a UTI.<sup>75</sup> Additionally, UTIs during gestation are reported to reduce litter size by 0.6 piglets/litter.<sup>76</sup> Sows diagnosed with a UTI had 3.5 times higher risk of developing endometritis compared with healthy animals.<sup>63</sup> Furthermore, UTIs are associated with other diseases, such as mastitis metritis agalactia.<sup>77-79</sup>

The UTI etiology is complex, polymicrobial, and may feature rotation or changes in etiological pathogens. Among the possible organisms, *E coli* was the predominant microbiological organisms isolated in single (71%) and mixed (85%) UTIs in sows.<sup>80</sup> For a long period, the urine within the urinary tract was generally considered sterile.<sup>81,82</sup> This was due to insensitive identification for

most bacterial species using traditional microbiological cultures.<sup>83-87</sup> However, a growing list of studies using DNA methods (PCR, NGS, and genome sequencing) detected a wide range of microbiological species in urine samples from diseased and healthy humans and animals.<sup>22,84</sup> Furthermore, it was observed that not only was DNA present, but that the bacterial strains were viable.<sup>86</sup> Therefore, the urinary bladder has an active and functional microbiome and may affect the onset of a UTI. The microbiome role in UTIs was demonstrated by a study in humans that administered *Lactobacillus crispatus* in vaginal suppositories after completion of a full course of antibiotic therapy, which reduced the recurrence of UTIs by 50% in UTI-prone women.<sup>19</sup> This is of particular importance in pigs because UTIs are prevalent in swine herds, and are usually treated with in-feed, broad-spectrum antimicrobials.<sup>88,89</sup> Another alternative for reducing the prevalence of UTIs, and consequently antibiotic use, is the use of urine acidifiers in the diet. The use of acidifiers affects the acid-base balance of the sow diet and is correlated with urinary pH and reduced total bacteria colony-forming units in the urine.<sup>90</sup> Similar results were found in a mouse model with the reduction of uropathogenic *E coli*.<sup>91</sup> Kluge et al<sup>92</sup> showed that supplementation with 1% benzoic acid in the diet reduced the urinary pH of sows by up to one unit when compared to the nonsupplemented group.

Few studies in animal science have analyzed the urinary tract microbiome. One study using dogs as a model identified a urinary tract microbiome in these animals.<sup>22</sup> There seems to be a relationship between vaginal and urinary tract microbiomes in animals and humans.<sup>19,22</sup> Similarly, a positive correlation between UTI and endometritis was observed in pigs.<sup>63,75</sup> Overlap between vaginal and urinary microbiota exists in dogs and humans, but more research is needed to determine if this overlap also exists in sows.<sup>19,22</sup>

However, there are no studies to our knowledge that have evaluated the urinary tract microbiome in sows and its relationship with the use of nutritional management strategies (eg, probiotics and acidifiers). Nevertheless, Xu et al<sup>53</sup> observed that lysozyme administration in sow feed altered vaginal microbiota. Other literature indicates that nutritional changes led to a reduction in urinary pH and a reduction in some potential

pathogens in sow urine.<sup>90,92</sup> If gut microbiome can be modulated to prevent dysbiosis, perhaps similar strategies can be used to prevent or even treat UTIs and consequently reduce the use of antibiotics. However, further investigation is necessary to understand the microbiome role in the sow bladder during cystitis and to develop new technologies and strategies to modulate the microbiome, minimizing dysbiosis and diseases.

## Colostrum and milk microbiomes

Besides their nutritional value, colostrum and milk are essential to stimulate immune system development of piglets.<sup>32,93-95</sup> Postpartum dysgalactia syndrome is commonly associated with infectious pathogens and is classified as having a multifactorial etiology. Postpartum dysgalactia syndrome compromises milk production and is triggered by associations between risk factors such as management, feeding, and hygiene.<sup>77-79</sup>

It was observed that a lack of sufficient milk production resulted in an increase in piglet preweaning mortality, especially during the first week of age where mortality can be up to 38.6%.<sup>79,96</sup> The infection of mammary glands may lead to their lack of function and impairment of pregnancy rate.<sup>79</sup> Mastitis has a complex treatment and, consequently, it was observed that a high percentage (23%-33%) of antimicrobials used were classified as highest priority or critically important for human medicine by the World Health Organization.<sup>12,97</sup> Moreover, Jenny et al<sup>97</sup> showed that for antibiotic treatment of sow mastitis, duration was shorter and dosage was lower than recommended in 54% and 19%, respectively, which can influence antibiotic resistance selection.<sup>96</sup> Based on the negative impact of PDS on reproductive performance and antimicrobial resistance, alternative tools are essential to reduce the occurrence of this syndrome.

The origin of colostrum and milk microbiomes is complex and not fully elucidated.<sup>98</sup> The high percentage of anaerobic intestinal microorganisms in milk samples indicates that part of the milk bacterial community originates from the maternal gastrointestinal tract through the bacterial entero-mammary pathway<sup>99</sup> or ascending colonization of the udder via the teat canal (galactogenic route).<sup>77,78,100</sup> Other studies indicate that the skin may also be a source for the colostrum and milk microbiome.<sup>101,102</sup> Bacteriological

analysis of colostrum and samples from mammary gland skin from healthy sows showed that all skin samples were bacteriologically positive with Staphylococcaceae as the most frequently isolated (96.9%) followed by Streptococcaceae (63.5%). In addition, 66.7% of all skin samples had species from the Enterobacteriaceae family, with *E coli* the dominant species. Similarly, 79.2% of colostrum samples were bacteriologically positive with Staphylococcaceae as the most frequently isolated (54.1%) followed by Streptococcaceae (30.3%) and Enterobacteriaceae (3.9%). Again, *E coli* was the dominant species among the Enterobacteriaceae family.<sup>102</sup>

Despite not fully understanding the makeup of the mammary gland microbiome, it was observed that sow milk contained Enterobacteriaceae<sup>102</sup> and anaerobic gut-associated genera such as *Bacteroides*, *Blautia*, *Ruminococcus*, and *Bifidobacterium* indicating that the gut has an essential role in the mammary microbiome composition.<sup>95</sup> Gerjets et al<sup>103</sup> studied the virulence genes most frequently detected in milk samples from healthy sows and sows with coliform mastitis. Although sows with coliform mastitis had significantly more specific virulence genes in their samples, healthy sows showed frequencies close to and even higher of some virulence coding genes.<sup>103</sup> Furthermore, no pattern was found in the virulence profile comparing sick and healthy animals.<sup>103</sup> These findings raise the question whether the presence of virulence genes alone is sufficient for bacteria to cause disease. There is no doubt that virulence genes are determinant for bacteria to attach, invade, and colonize the host resulting in illness.<sup>104</sup> However, it also indicates that there is a complex interaction among pathogenic and opportunistic organisms, the environment, and animal genetics. The disruption of one of these factors by stressful handling, mixing of animals from different origins, or the entry of a new infectious pathogen in the naive herd can affect the microbiome allowing the multiplication of pathogenic bacteria causing dysbiosis and disease.

Chen et al<sup>95</sup> analyzed the bacterial 16S rRNA gene sequences from sow colostrum and milk, and the predominant phyla were Firmicutes and Proteobacteria with a counter-balanced relationship between them. The relative abundance of these two phyla significantly fluctuated throughout lactation, while total

proportions between them remained at a certain level (75.9%-80.9%).<sup>95</sup> The predominant genera observed during a microbiome assay was different between sow colostrum and milk. The most predominant genus in the colostrum was *Streptococcus*, while transitional and mature milk samples were dominated by unclassified Ruminococcaceae, *Bifidobacterium*, *Staphylococcus*, and *Acinetobacter*, which are lactose-utilizing genera.<sup>95</sup> The six most predominant genera in sows' milk were Ruminococcaceae, *Streptococcus*, unclassified Clostridiales, *Lactobacillus*, *Corynebacterium*, and unclassified Lachnospiraceae.<sup>95</sup> Analysis from bacteriological isolation<sup>102</sup> and 16S rRNA sequences<sup>95</sup> indicates that *Staphylococcus* and *Streptococcus* are generally the predominant genera in sow colostrum and milk. Moreover, it was reported that microbiome changes in the mammary gland can be the cause for some nutritional alterations from colostrum to transitional and mature milk.<sup>95</sup>

It was observed that microbiome in the gut is related to diseases in other organs<sup>41,53</sup> and a probiotic/prebiotic or symbiotic supplementation may reduce the shedding of potential opportunistic organisms.<sup>37,53</sup> The bacterial entero-mammary pathway is being established<sup>99</sup> and this interconnection indicates that gut microbiome modulation may affect colostrum and milk microbiome composition. In this context, lysozyme feed supplementation altered fecal microbiome and decreased some proinflammatory and increased anti-inflammatory cytokines. These inflammatory cytokines may play a role in PDS development.<sup>105</sup> Based on this, the mammary gland microbiome and its interaction with the gastrointestinal microbiome would constitute an alternative strategy to prevent mammary disorders through gut microbiome modulation and consequently reduce the use of antimicrobials to treat mastitis. Another possibility to reduce the occurrence of mastitis is the development of probiotics for topical application to the sow udder to exclude opportunistic organisms from colonizing the mammary gland. Similar strategies using probiotics in the form of biofilm, spray, or intramammary inoculation to prevent mastitis have been developed and have shown promising results *in vitro*<sup>106</sup> and in dairy cows.<sup>107,108</sup> Furthermore, formulations to be applied in sows should also be beneficial to piglet gut health.

Finally, the sow colostrum and milk microbiome can also influence piglet gut development and innate immune response. The maternal milk microbiome is primarily responsible for the colonization of the piglet gut contributing approximately 90% of the bacteria throughout the first 35 days of life.<sup>32</sup> *Lactobacillus reuteri*, *Lactobacillus mucosae*, and *Akkermansia muciniphila* are present in sow milk and can act as potential probiotic bacteria.<sup>109,110</sup> An increase of these organisms in the milk was observed during the lactation period.<sup>95</sup> Conversely, potentially pathogenic bacteria such as *Staphylococcus epidermidis*, *Helcococcus*, *Corynebacterium*, *Actinobacillus*, and *Haemophilus* are also present in sow milk, but these organisms generally decreased during lactation in healthy sows.<sup>95,111,112</sup> The *Helcococcus* genus was negatively correlated with the abundance of the most bacteria genera in sow milk<sup>95</sup> and its increase in the milk may affect sow and piglet health.

Further studies exploring the sow milk microbiome are necessary to determine a microbial core. More research is also needed to evaluate the influence of environmental characteristics and the gut microbiome on the colostrum and milk microbiome and the subsequent impacts on the offspring.

## Fecal microbiome and reproduction

The increased number of piglets born with lower birth weights and the greater within-litter weight variation leads to concerns about the ability of the sow to satisfactorily raise the piglets until weaning. In recent years, numerous studies were developed to understand the impact of the sow gut microbiome and the effects of microbiome modulation on offspring performance. Moreover, the gut microbiome has been studied to find possible biomarkers for productivity, and studies related to FMT were conducted to observe the impact of microbiome of different genetic lines on productivity.

The colonization of the piglet gut is initiated during the farrowing process and immediately after birth. This early colonization plays a crucial role in intestinal maturation. The developmental process of the intestinal microbiome is similar for humans and most animals.<sup>113</sup> The earliest colonizers in the gut are facultative anaerobes, which are responsible for the creation of a favorable environment for anaerobe establishment.<sup>114,115</sup>

Chen et al<sup>115</sup> demonstrated that the core microbiome of piglet feces in the first days post partum is determined by surrounding environmental factors such as floor microorganisms and the microbiomes of the sow's vagina, teats, mammary secretions (colostrum and milk), and feces. Also, several studies demonstrated that the process of immune maturation is influenced by the microbiome that colonizes the gut during the early stage of life.<sup>116,117</sup> The piglet gut microbiome is influenced by milk oligosaccharides (MOS). The MOS decrease intestinal pH and increase cecal and colonic butyrate in the piglet gut and have prebiotic activity, anti-adhesion effects, and anti-inflammatory properties. These characteristics stimulate the growth of beneficial microbes and inhibit possible pathogens.<sup>118,119</sup> It was observed that sows fed with chitoooligosaccharide supplement had altered MOS with increasing trisaccharide and tetrasaccharide, but the impact on the piglet gut microbiome was not evaluated.<sup>119</sup> Although a plethora of preweaning and postweaning factors (eg, tail docking, teeth clipping, antibiotic treatment, weaning-associated stressors, and diet composition) may affect the gut microbiome of piglets, a maternal influence on the piglet microbiome was observed for up to 63 days of age.<sup>120</sup>

Dysbiosis in the intestinal microbiome may increase gut permeability and plasma endotoxin concentrations leading to sow metabolic disorders and exacerbated inflammatory status during early lactation.<sup>121</sup> Wang et al<sup>27</sup> found that differences in the intestinal microbiome of sows resulted from oxidative stress during the peripartum period. The authors observed that the relative abundance of *Bacteroides* was correlated to a reduced dam oxidative stress status and higher litter weight on day 21 of lactation. In contrast, *Phascolarctobacterium* and *Streptococcus* were associated with increased oxidative stress and lower litter weight at 21 days post partum.<sup>27</sup>

In highly productive sows, the gut microbiome at 3 days before farrowing was mainly enriched in genera belonging to the Prevotellaceae and Ruminococcaceae families and a relative abundance of gram-negative bacteria in comparison to sows classified with low productivity.<sup>4</sup> Sows classified as high performing during gestation<sup>15,122</sup> and lactation<sup>27</sup> had lower microbiome diversity. Uryu et al<sup>15</sup> also identified that sows with high reproductive performance had an increase in

the relative abundances of 43 bacterial genera, markedly the short-chain fatty acid (SCFA)-producing bacteria.

One important factor to evaluate during gut microbiome manipulation is SCFA production. The SCFAs play a role in sow metabolism, immune regulation, and gut homeostasis<sup>31,122-124</sup> and act as precursor of colostrum and milk fat.<sup>125</sup> Moreover, the SCFA-producing bacteria were negatively correlated with porcine epidemic diarrhea virus infection<sup>23</sup> and heat stress.<sup>30</sup> Brutsaert<sup>126</sup> indicates that feeding the sow with a nutritional additive (phenolic compound, slow release C12, target release butyrate, medium-chain fatty acids, and organic acids) has the potential to stabilize the sow gut microbiome during parturition, increase feed intake, and increase the proportion of females that produce heavier piglets at weaning.

The fermentation of dietary fiber, notably soluble fiber, by the hindgut microbiome leads to high production of SCFA<sup>124</sup> and improves piglet development,<sup>127</sup> reduces pathogenic bacteria in the gut,<sup>123,124</sup> reduces digesta transit time, and may prevent colonization by opportunistic organisms and lipopolysaccharide absorption.<sup>128</sup> According to Jiang et al,<sup>43</sup> sows that received a diet with 7.5% crude fiber throughout the reproductive cycle, as compared to sows that received 2.5%, had an increased litter size (3.57 piglets/litter), increased proportion of genera considered beneficial to the intestinal microbiome (*Ruminococcus*, *Butyrivibrio*, *Lactobacillus*, and *Fibrobacter*), and decreased potentially pathogenic genera such as *Clostridium*, *Streptococcus*, *Bacteroides*, and *Escherichia-Shigella*. When the level of dietary fiber was the same, a higher soluble fiber vs insoluble fiber inclusion improved enzymes with antioxidant capacity and decreased proinflammatory factors in the sows and their offspring.<sup>129</sup> The authors also reported that soluble fiber in sow diets increased the proportion of *Romboutsia*, *Sediminibacterium*, *Bifidobacterium*, unidentified Lachnospiraceae, unidentified Ruminococcaceae, *Subdoligranulum*, *Bacillus*, *Blautia*, *Bacteroides*, and *Parabacteroides* and reduced the proportion of *Acinetobacter*, *Vagococcus*, and *Streptococcus* in sow feces and piglet colons.<sup>129</sup> The microbial organisms reduced in the piglet colon were already characterized as opportunistic organisms.<sup>130-132</sup> Similarly, Cheng et al<sup>133</sup> observed that increasing soluble fiber to 2% in the sow gestation diet resulted in piglets with

greater growth rate and lower diarrhea rate during the lactation period. Furthermore, the inclusion of dietary fiber in sow diets may contribute to maintenance of proper satiety throughout gestation,<sup>134</sup> reduced constipation,<sup>128</sup> decreased farrowing duration,<sup>127</sup> and reduced stillbirth rate.<sup>125</sup>

Supplementing the diet with functional foods capable of altering the intestinal microbiome has also been an area of research in recent years. Hasan et al<sup>29</sup> showed that the supplementation of yeast hydrolysate in sow diets changed the composition of the fecal microbiome of pregnant sows at the phylum level, reduced farrowing duration, and increased colostrum production, which resulted in a 13% increase in colostrum consumption by piglets. In addition, a lower relative abundance of the phylum Proteobacteria was observed in the supplemented group, which can be considered beneficial since the increased prevalence of this phylum is a marker of dysbiosis associated with intestinal diseases and inflammation.

It is well established that nutrition during the rearing period may affect the performance of future gilts<sup>135</sup> but there is a lack of information regarding the gut microbiome role in this aspect. Emerging evidence in rats suggests that the gut microbiome may affect reproductive function since estrogens interact with the commensal microbiome through the estrogen-gut microbiome axis.<sup>136,137</sup> Wang et al<sup>138</sup> observed that the gut of gilts showing failure to enter estrus before 210 days of age was enriched with Ruminococcaceae, Lachnospiraceae, *Ruminococcus*, *Coprococcus*, and *Oscillospira*. In contrast, gilts showing a normal heat cycle had higher abundance of *Prevotella*, *Treponema*, *Faecalibacterium*, *Oribacterium*, *Succinivibrio*, and *Anaerovibrio*. In the same study, the authors found that the abundance of both *Sphaerochaeta* and *Treponema* was associated with specific periods of the estrus cycle in which estrogen is high (estrus and proestrous).

Some studies showed that most of the afore mentioned genera may be increased in the gut microbiome of sows and gilts by including fiber in the diet.<sup>123,133</sup> The high inclusion of fiber, predominantly soluble (50% beet pulp), between the 1<sup>st</sup> and 19<sup>th</sup> day of the 3<sup>rd</sup> post-puberty estrous cycle resulted in improved oocyte quality and embryo development *in vitro* and *in vivo*.<sup>139,140</sup> Also, the inclusion of 350 g/kg of lupine

(rich in insoluble fiber and a moderate amount of soluble fiber) in the diet of prepubertal gilts improved oocyte quality and embryonic survival at 28 days of age. Moreover, a recent study showed that highly prolific Meishan sows have increased fecal microbiome diversity and levels of fecal steroid hormones (estradiol and progesterone) than less prolific sows, which may contribute to the improvement of sow reproductive performance.<sup>29</sup> Xu et al<sup>141</sup> observed that the gut of sows with a short wean-to-estrus interval had lower *Prevotella* and *Bacteroides* at the genus level, whereas Firmicutes and Lentisphaerae are greater at the phylum level.

The uterus of Meishan gilts secrete more endometrial proteins than the uterus of white crossbred gilts and that the secretion of endometrial proteins is positively correlated with endometrial gland development before 60 days of age. Xu et al<sup>28</sup> designed a study to evaluate the role of the gut microbiome on endometrial gland development through an FMT from Meishan to Landrace × Yorkshire gilts from 90 days of age until puberty. Fecal microbiome transplantation explained 60.49% of the variation in gut microbiome and increased concentrations of SCFAs, endometrial gland area, insulin-like growth factor 1 (IGF-1) concentration in plasma and uterine tissue, and mRNA expression level of estrogen receptor 1 gene in ovary tissue. The authors also observed that Lentisphaerae, *Bifidobacterium*, and *Fibrobacter* were positively correlated with endometrial gland area; Bacteroidetes was negatively correlated with estradiol and IGF-1 concentration; Firmicutes and *Fibrobacter* were positively correlated with estradiol concentration; and Bacteroidetes was positively correlated with progesterone concentration while Fibrobacteres, Firmicutes, *Bifidobacterium*, and *Fibrobacter* were negatively correlated.

## Conclusion and future approaches

The microbiome composition is very sensitive and influenced by diverse environmental, management, and nutritional events. Recent studies indicate that in some cases correlations are insufficient to understand the microbiome complexity. The productivity of offspring may also be affected by sow microbiome modulation. Sow microbiome modulation with probiotics, prebiotics, symbiotics, or other feed additives or nutritional

management may constitute a new tool to increase productivity and reduce disease in swine herds and consequently reduce antimicrobial use. Some biomarkers for productivity and disease have been identified, but further investigation using different herds are necessary to determine causality and repeatability of these findings. Future studies should focus on system biology approaches to understand the microbial-microbial and microbial-sow interactions as well as the effect of microbial metabolic production on reproductive outcomes and disease. Randomized blinded clinical trials are necessary to determine if it is possible to increase or decrease target microbial genera previously identified as biomarkers in metagenomics studies and their impact on reproductive outcomes and disease. The decrease in cost per base sequenced over the past few years is encouraging further research in this area. With an increase in metagenomics studies, future research may be aimed at the development of more specific and useful commercial products and to guide future genetic selections.

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## Conflict of interest

None reported.

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