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Open document on the priority list of factors affecting the occurrence of dysbiosis in the GIT of pigs as prerequisite to define a road map to improve the pigs' health

The gut dysbiosis is a multifactorial event due to a cascade of occurrences that determine the imbalance of the gut microbiota, which may lead to severe enteric diseases. In the industrial pig production, the life of pigs is divided in growing phases, from suckling to growing-finishing or reproductive phase. The pigs from 30 kg of body weight are considered completely developed both physiologically and immunologically. The resistance of these animals to the dieses is determined by the management and dietary strategies adopted. Anyway, their robustness could be determined already in the early stage of their life by affecting the physiological development programming. In this context, the early colonization of the GIT by beneficial microbes is now considered of utmost importance to drive the early physiological and immunological maturation of the pig. Moreover, the early imprinting seems to have a long-term effect along the whole life of the animals. In this context, the PiGutNet-network prioritized to discuss and deepen the exchanges on the young animals. In fact, an impairment of their health status can compromise the whole development of the animals with long-term negative effect on the pigs' health by a general impairment of their robustness.

Taking advantage from the interdisciplinary competences in the network of scientists and companies, a list of the most important factors able to favor or predispose the animals to the gut dysbiosis were highlighted, focusing the attention on the pre and post-weaning phase of the pig:

Pre- weaning: The suckling period, from birth to weaning, is a key period in which is possible to affect the physiological development of the pigs with effect along all the pigs' life. PiGutNet identified the following points:

- *Birth body weight*: The birth body weight is a key point that need to be considered to globally improve the health of the litter. The increased number of pigs per litter due to the selection for high prolific sows, poses some major challenges in practice: The low body weight (LBW) pigs represent an intra-litter sub-population that increase the complexity in properly prepare all the pigs for the weaning. Because the weaning weight is related with the post-weaning survival, it is important to favor the weight recovery of the LBW pigs by adopting management strategies (e.g. cross fostering) and appropriate feeding strategies (e.g. provision of milk replacers and appropriate creep feed) during the suckling period. From the other hand, all the strategies oriented in prevent the number of LBW pigs are essential to improve the robustness of the animals reducing risk of disease occurrence. Among that, feeding strategies for gestating sows (e.g. Arginine), genetic selection to increase the litter weight as well the litter weight homogeneity, should be considered.

- *Colostrum*: Newborn pigs require to suckle in the first 24 hours, and at least 100 ml colostrum is required (Devillers et al., 2011). The immunological function of the colostrum is well-known, anyway the colostrum is a complex feed that contains, together with essential nutrients, several growing factors and bioactive compounds (e.g. peptides, oligosaccharides, fatty acids) that are essential for the physiological maturation of the piglets. The two main factors to consider are the timing of ingestion and the composition of the colostrum. Both these factors are essential to guarantee the gut health of the pigs and consequently a

proper development of the young animals. The modulation of the colostrum quality by feeding the sows with specific additives is an important area of research that needs to be deeply explored.

- *Genotype*: The attention on the pig robustness is growing quite fast, particularly in relation with the demand to identify alternative to the use of antimicrobials in the livestock production. The application of selection indexes considering directly or indirectly the survival of piglets, such as additional body weight on live piglets at 21 days age (Martinsen, 2016) may contribute to a better robustness of the gut microbiota, because of the general improved condition of the subjects. However, this response has not received a lot of research attention yet within the academic society and the industries. The growing knowledge of the pig genome and the inclusion into breeding schemes of some specific markers related with the interaction between host and bacteria in the pig selection (e.g. resistance to E. coli infection), can contribute to reduce the risk of gut dysbiosis from suckling to weaning pigs.

- *Environmental stressors*: newborn pigs are physiologically and immunologically underdeveloped. The loss of body temperature due to an improper micro-environment in the farrowing unit and the retardation in the colostrum assumption, which provides energy to the newborn pig, can impair the health of the piglets, with primary effect on the GIT. Especially in the LBW pigs, the risk of diarrhea occurrence is high during suckling. Thus, the proper design of the nest where the interaction of the newborns with the sow takes place is particularly relevant.

- *Management*: animal welfare is a new or re-discovered area of research to prevent the health impairment of the animals. Pigs, even during the first days of life can be subjected to several manipulations like tail docking, castration and Fe or antibiotic injection. All these practices, increases the animal discomfort as well generate local or systemic inflammation that can impair the physiological development of the animals or can predispose the animals to infections. The washing of sows when entering in the farrowing room is a practice that can control the transmission of some environmental intestinal pathogens (like Coccidia and certain Clostridia); this could indirectly favor the establishment of intestinal eubiosis in piglets. In addition, cross-fostering of piglets is a diffused practice. The fostering of LBW pigs, particularly from litters of larger sizes, within 24 hours after birth, is considered in general a strategy leading to improved fitness. Thus, it can be supposed that this reduces the risk of their intestinal dysbiosis. Nevertheless, piglets will carry the intestinal bacteria that they derived from their natural mother into the new litter and the implication for the balance of the microbial community inside the new formed group has not enough been explored.

- *Antibiotics*: during suckling, the use of antibiotics should be limited thanks to the protective effect of the passive immunity provided from the colostrum and milk. Anyway, there are some specific conditions that increase the risks of health impairment during suckling, among those, the prevalence of LBW pigs is a risk factor that can provoke dysbiosis early post-birth. In addition, some procedures like castration and tail docking can predispose infection with the consequence need of antibiotic administration. The administration of antibiotics in the early life of pigs can have a long term effect on the gut microbial structure with consequences along the whole life of the pigs.

- *Diets*: Among the feeding strategies, the formulation of milk replacers and/or creep feed is an important area of research, especially for the high prolific sows due to the high frequency of LBW pigs per litter. The efficacy of the administration of these products is still debated in the world, notwithstanding a large research literature, mostly done however on litters of moderate size, because obtained with more traditional genetic lines. In general, the objective of these products is to sustain the piglet's growth.

Anyway, the secondary objective is to provide nutrients able to sustain the gut health by supporting the colonization of the gut by beneficial microbes, to improve the mucus barrier and the maturation of enterocytes (e.g. insoluble fiber, some AA as arginine, threonine, glutamine). The improper formulation of these feeds can provide a substrate for the overgrowth of pathogens increasing the risk of gut dysbiosis in newborn.

Post-weaning: In the intensive pig production, post-weaning period is a critical stage in the animals life. Even if strategies are adopted during the suckling period to improve the host maturation, pigs are not completely developed to digest vegetal substrates as well for its immune competence at weaning (28 days of age) the. This condition, together with the so called 'weaning stress' due to a plethora of social and environmental changes, can predispose the piglets to dysbiosis. PiGutNet identified the most important risk factors that can predispose an imbalance of the gut microbiota.

- *Diets*: It is commonly reported that the diet is the first environmental factor able to affect the gut microbiota in the short time. The changes in protein, fat and carbohydrates composition of the diets act as selective factor, modifying the substrate available for the microbial growth, affecting the microbial profile and/or its function. In order to prevent the gut dysbiosis, especially in the post-weaning phase, feed additives are commonly used with the intention to modulate the gut microbiota profile reducing the risk of dysbiosis. Feed additives and some minor feed ingredients can exert several functions (e.g immune stimulation, improve feed digestion, antioxidant effect). For this purpose, the research of new additives is one of the most active research areas, especially to replace antibiotics. A special focus is the use of zinc oxide (ZnO) that is supplied at a high dosage, and this strategy is today the most used supplement as alterative to the antibiotics to counteract gut dysbiosis at weaning. However, apart the environmental issue, already highlighted for the EU Commission (in EU the high dose of ZnO will be banned in 5 year), the continuous use of ZnO associated to the administration of certain antibiotics, seems to exacerbate the occurrence of antimicrobial residence (AMR) (Vahjen et la., 2015). For this reason, the EU support projects to develop new non-antibiotic additives.

- *Age at weaning*: especially in the intensive farming production, where the average weaning age is 24-26 days of age, the risk related to the occurrence of post-weaning dysbiosis is high (Gresse et al., 2017). The most critical factor is related to the physiological immaturity of pigs, especially the development of the immune system. In fact, piglets reach their complete immune competence around the 7th week of age. At weaning, the supply of immunoglobulin A from milk is suddenly stopped and between the 4th and 5th week of age, the local passive immunity conferred by the colostrum decreases. Considering that at weaning a strong modification of the GIT microbiota is reported, mainly due to the dietary changes and societal stress, the risk of dysbiosis is quite high, especially in sub-optimal sanitary conditions. Increasing the weaning age from 28 to 35 days of age can improve the natural resistance of the animals to the pathogens.

- *Weight at weaning*: The weaning weight is an important factor to estimate the potential of survival of the pigs in the post-weaning period. Low birth pigs (with good vitality) could had a reduced milk intake during lactation but they will be more able to digest the feed at weaning, because they will have already tried it (creep feed), so they could also be considered not bad pigs but only delayed pigs. On the other side, heavier piglets may be more PWD-susceptible because they had a higher milk intake during lactation, so they did not explore other feed sources. This can increase the weaning stress as well as delay the post-weaning feed intake and the digestion of the complex feed supplied at weaning, with the increasing risk of dysbiosis. All

factors oriented to increase the weight at weaning are of utmost importance to prevent health impairment of piglets.

- *Management*: weaning is the most stressful event in the pig's life. A proper management can reduce the discomfort of pigs, reducing the duration of the post-weaning transient anorexia that is a key factor in predispose the gut dysbiosis. Improving animal welfare, by reducing litter mixing, providing enough space and environmental enrichment, seems to have a positive effect on the pig health. The co-mingling of piglets from different litter a few days before weaning can be an interesting opportunity to dilute the weaning stress (Parratt et al., 2006). Studies are needed to reinforce the link between animal welfare and the gut eubiosis.

- *Genotype*: The risk of enteric infection can be exacerbated by the simultaneous exposure to a high pathogen load (e.g. enterotoxigenic Escherichia coli) in the farm, and pigs genetically susceptible to the pathogens may be at high risk to develop post-weaning diarrhea. In fact, especially for the most common pathogens responsible for the post-weaning diarrhea, E. coli F4 and E. coli F18, has been identified genetic variants able to provide resistance or susceptibility to this pathogens. From the other hand, genetic variants linked with a most wide crosstalk between host and bacteria (e.g TLRs, ABO, BPI) are identified. In this context it is clear that the genotype can affect the piglets response against gut microbiota, commensal and pathogens, and at the most it can modulate the gut eubiosi. The growing knowledge of the pig genome and the inclusion of some specific markers related with the interaction between host and bacteria in the pig selection (e.g. resistance to E. coli infection), can contribute in reduce the risk of gut dysbiosis or most in general increases its robustness especially for young animals.

- *Antibiotics*: Even though the AGPs are banned in Europe since the 2006, this substances are still largely used worldwide, as well the administration of antibiotics as therapeutics in post-weaning phase is frequently used to prevent the pathogen infection especially related with gut dysbiosis. Is reported that in livestock, part of the antibiotics are used for habitude without a diagnosis or before the results of a antibiotic susceptibility testing (antibiogram). The long term consequences of this habitude increasing the risk of the occurrence of AMR factor that reduce the possibility to counteract dysbiosis due to the lack of antibiotic efficacy. The AMR factor represents a long term modification of the bacterial genetic background and the epidemiology studies reported the increasing of the spread of AMR genes. Of course AMR is a complex matter of study but it is urgent to develop strategies to reduce the use of antibiotic in order to contain the occurrence of new AMR.

PiGutNet identified these points as essential to design new tools for improving the health status of pigs by reducing the risk of gut dysbiosis. Considering the inter-connection between all the phases of pig's life, from embryo development to adult life (continuity concept), it is evident that there is a need to develop an integrated strategy that pays attention as much as possible to all the risk factors listed above. It seems clear that the strategies could be divided in short-term (e.g. diet, management) and mid-long term (e.g. genetic) solutions. Hence, designing a roadmap should be considered to develop a synergistic strategy aimed at the best rearing practice as a whole. As the ultimate objective, this road map should be conducted to reduce the dependency of the farming system for the antibiotic use, in order to obtain a more sustainable pig production.

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