RECENT DATA ON THE PHYSIOLOGY, MICROBIOLOGY AND IMMUNOLOGY OF THE GUT OF PIGLETS AROUND WEANING. IMPLICATIONS FOR ALTERNATIVES TO IN-FEED ANTIBIOTICS

Jean-Paul Lallès[1], Sergey R. Konstantinov[2], Hauke Smidt[2], Hermann-Josef Rothkötter[3] and Chris R. Stokes[4]
[1]INRA-UMRVP, 35590 Saint-Gilles, France, [2] Wageningen University, Wageningen, The Netherlands,
[3] School of Medicine, Magdeburg, Germany, [4]University of Bristol, Bristol, U.K.

Introduction

Weaning is a critical phase for pigs. It is characterised by a drastic but transient reduction in voluntary feed intake, a growth check and an increased susceptibility to gastrointestinal disorders, pathogens and diarrhoea. The EU ban on in-feed antibiotics and severe restrictions in the use of metals including copper and zinc, makes this period especially difficult to manage. Changes in the architecture and functions of the small intestine have been well documented (1), and restoration of intestinal integrity occurs within one to two weeks.

In terms of microbiology, the gut is colonised at birth and the microflora develops from a simple and unstable to a complex and more stable community. At weaning, major changes happen in the microflora composition as influenced by the diet and environment (2, 3). Developments in this field have been important over recent years, especially towards the non-cultivable bacteria, thanks to molecular techniques. At birth, the piglet is immunologically naïve and is dependent on the passive transfer of immunity from the sow to the piglet through colostral immunoglobulins. By contrast, the development of active immunity, that is the capacity to become tolerant to dietary antigens and commensal bacterial together with the ability to mount appropriate immune responses against pathogens, is still poorly understood (4).

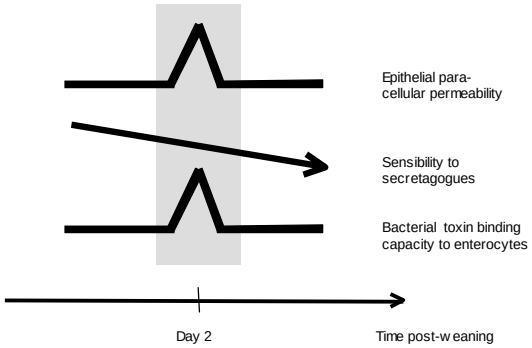
Here we report recent data obtained in intestinal physiology, microbiology and immunology in young pigs and some implications in the dietary management of weaning without antibiotics.

Intestinal physiology

The intestine executes various functions including digestion and absorption of nutrients, absorption-secretion of minerals and water, secretion of mucus, and epithelial barrier against pathogens and noxious substances. Villous atrophy and crypt hyperplasia of the proximal small intestine together with longitudinal alterations in digestive enzyme activity profiles have long been recognised as the hallmark of weaning (1). Several years ago it was hypothesised that an allergy to dietary proteins was the cause of these changes but more recent data suggest that post-weaning anorexia may also play a role (5).

Weaning has marked effects on the expression of cell protection systems such as heat shock proteins (HSP), with regional and temporal specific patterns (6). For example, the over-expression of HSP27 and HSP70 was precocious and more pronounced in the proximal segments of the gut. By contrast, HSP90 expression was unchanged or reduced, this happening later and more distally along the gut. *In vitro* studies with Ussing chambers recently revealed a hyper-secretory state of the mucosa of the proximal small intestine and colon 2 to 4 d post-weaning (7, 8). This was also associated with a transient increase in small intestinal paracellular (tight junction) permeability. By contrast, the intestinal secretory capacity to secretagogues, as stimulated with various bacterial toxins, was found to decrease with time throughout weaning (8) (figure 1).

Figure 1. Changes in intestinal physiology over time post-weaning in pigs (G. Boudry, unpublished).



Collectively, these data highlight the deep disorders in intestinal architecture and functions immediately post-weaning. They are probably related to the transient anorexia since many of them occur following fasting, as shown in various animal species. This acute period is then followed by the acquisition of a gastro-intestinal tract of adult-type anatomy and functions.

Intestinal microbiology

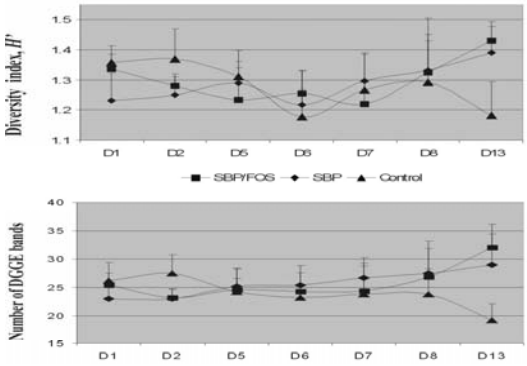
In pigs, the development of the intestinal microflora follows a rapid succession between birth and weaning, but it is relatively stable and dominated by lactic acid bacteria as long as piglets are kept suckling. However, marked qualitative and quantitative changes appear with solid feed consumption. For example, strict anaerobes such as *Bacteroides* establish in the large intestine, at a time when the number of facultative anaerobes is declining (9). In connection with health, some intestinal bacteria are known for their pathogenicity (*Clostridia*, *Salmonella*, etc.), others for their protective effects (*Lactobacilli*, *Bifidobacteria*), and some having variable influences depending on local conditions (*Escherichia coli*, *Bacteroides*, etc.). Modern molecular microbiology techniques have been applied recently to study the ecology of the porcine gastrointestinal tract. They have provided new insights into bacterial colonisation and its temporal changes, especially as far as the non-cultivable bacteria are concerned (10).

The effect of diets with two levels of fibre and with or without antibiotics was studied throughout weaning (11). Combining classical and molecular techniques revealed intestinal dominance of *Lactobacilli*. Irrespective of the diet, they were transiently depressed after weaning. Most of these *Lactobacilli* corresponded to *L. amylovorus* previously detected in Danish pigs (12).

The influence of dietary carbohydrate fermentability on the gut microflora was also investigated. Piglets consuming a diet supplemented with sugar beet pulp alone or mixed with fructo-oligosaccharides (FOS) showed a faecal bacterial community that was more diverse and more stable than the controls fed the non-supplemented diet (13) (figure 2).

Numerous bacterial species identified using molecular techniques did not correspond to those obtained by cultivation. For example, 16 rRNA gene sequences related to *Ruminococcus spp.* were detected in the colon of all the supplemented piglets but not in the controls (13). Thus, these non-cultivable bacteria would probably play an important role in the fermentation of dietary fibres in weaned piglets. In a similar manner, the responses of the gut bacterial communities upon the addition of inulin, lactulose, wheat starch and sugar beet pulp were analysed. The results indicated that the dietary addition of these fermentable carbohydrates support the growth of certain lactobacilli in the upper part of the intestine and lead to a higher bacterial diversity in the colon (14). Specifically, it was found that *L. amylovorus*-like populations were prevalent in the gut of the piglets fed with these fermentable carbohydrates. Such stimulation of the *Lactobacillus* community within the gastrointestinal tract of piglets may be of specific importance because of their possible antagonistic activities towards intestinal pathogens, a process termed "colonization resistance". Since a stable and complex commensal bacterial community is a prerequisite of a healthy gut ecosystem (15), the promotion of colonization resistance through the addition of fermentable carbohydrates (prebiotics) may be a comparatively easy way to improve enteric health at stressful times, such as early and abrupt weaning as experienced by piglets within a production environment (16).

Figure 2. Influence of supplementation of the diet with fermentable carbohydrates on bacterial diversity over time post-weaning in the faeces of piglets (13).



Collectively these data illustrate rapid succession at the time of weaning, and that some dietary formulas may improve the microbial balance, ensuring normal animal growth and nutrition of weaning piglets.

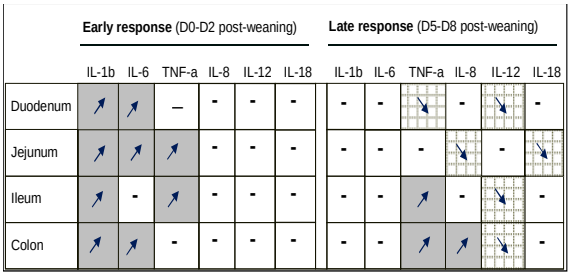
Intestinal immunology

The first immune line of gut protection after birth is passively derived IgA, because a passive transfer of maternal IgG during gestation is not possible as the pig has a six layered placenta. However, more complex immune pathways are required for the development of appropriate active immune responses and for the maintenance of gut epithelial integrity.

The immune system of the young pig is immature at birth, probably reflecting the lack of exposure to antigens. Then the structural development of the gut immune system depends on antigen exposure (17) for while at birth, the intestine is virtually devoid of lymphoid structures, the Peyer's patches develop during the first two weeks and the intestine is rapidly colonised with immune cells. Between weeks 2 and 4, CD4+ T cells appear in the lamina propria together with B cells expressing IgM. Cytotoxic CD8+ T lymphocytes in the epithelium and IgA+ B lymphocytes in the lamina propria appear from week 4 of age onward (18). Thus an adult-type intestinal immune system is reached by approximately 7 weeks of age. The piglet is able to mount active immune responses against viruses and dietary antigens from week 3 onward (4) but it acquires oral tolerance to dietary antigens only after week 8 (19). This inability to regulate immune responses to harmless antigens might contribute to post-weaning diarrhoea (20). Functional aspects, including intra-epithelial lymphocyte responses to mitogens and ability of T lymphocytes to secrete IL-2, have been shown to be transiently reduced at weaning (4). Also atypical (CD2+) cell types accumulate in the lamina propria, probably indicating a relocalisation of circulating immune cells to the gut (17). The dendritic cell network in the lamina propria and the Peyer's patches provide the basis for adequate antigen presentation. So far the development and function of these cells at the period of weaning are not known yet.

Recent investigations showed that after weaning, piglets express mRNA for both inflammatory (IL-2, IFNɔ, IL-12p40) and anti-inflammatory (IL-4, IL-5, IL-10) cytokines in all intestinal segments (E Sowa & HJ Rothkötter, unpublished). Inflammatory cytokines play a key role in inflammatory processes of the intestine, as shown in various laboratory animal species. In piglets, weaning is associated with intestinal inflammation (5). We studied in detail this period from the cytokine gene expression perspective (21).

Figure 3. Influence of weaning on intestinal cytokine gene expression in piglets (21).



During the first 2 days post-weaning, we observed increased levels of mRNA expression for IL-1ɓ, IL-6 and TNF-Ŭ along the small intestine and proximal colon (fig 3). This inflammation was contemporary with intestinal villous atrophy and reduced digestive enzyme activities of the mucosa (21). Then, expressions of IL-1ɓ and IL-6 returned to pre-weaning levels while that of TNF-Ŭ was still high in the ileum and colon. This might be related to post-weaning development of fermentations in the large intestine. Finally, mRNA expressions of IL-12 and IL-18 were not changed up to 2 days post-weaning but they were reduced thereafter, reaching probably adult-type profiles (21).

Feeding fermentable carbohydrates to piglets post-weaning favoured the reduction of IL-6 and IL-12p40 mRNA expression but it had limited impact on that of IL-1ɓ (IP Oswald *et al*, unpublished).

Therefore, beside the ontogenetic development of the local immune system of the pig, the gastro-intestinal tract displays

important changes in the gene expression of cytokines throughout weaning. The role of immune effectors in intestinal tissue alteration and restitution remains to be investigated.

Some dietary implications

Whilst post-weaning anorexia is often considered to be a primary factor in the aetiology of gut disorders in piglets (5), other factors stimulating early voluntary feed intake must contribute to enhancing gut health through physiology, microbiology and/or immunology pathways. Many alternative substances to in-feed antibiotics have been studied thus far. Here we highlight some dietary factors of interest only.

Different studies have shown that feeding liquid, fresh or fermented, diets stimulates appetite and improves gut condition (22). This helps to maintain gastric pH below 4 with high levels of lactic acid, thus inhibiting the outgrowth of pathogens (*E. coli*, *Salmonella*) and promoting the development of yeasts (3). However, growth is usually lower with fermented feed, probably due to degradation of supplied free amino acids.

Another example is spray-dried plasma (SDP). Although being an animal protein source non-authorized in the EU, this product has been demonstrated to stimulate feed intake (+25%) and growth performance (+27%) (23). Incidence and severity of post-weaning diarrhoea together with intestinal alterations are usually reduced. Beside improved feed palatability, other factors including plasma growth factors (IGF-1), non-immunoglobulin glycoproteins and total and *E. coli* specific antibodies are probably implicated (23). SDP reduced the tissue expression of inflammatory cytokine genes and density of immune cells in the intestinal mucosa (24, 25). However, piglets supplemented with SDP displayed surprisingly elevated immune responses and increased intestinal tissue alterations following stimulation with bacterial lipo-polysaccharide (25).

Feed composition per se appears to have contrasting effects on gut health criteria immediately post-weaning (5, 7, 26). For energy, supplying either glucose, lactose or starch to weaner pigs did not make any difference on post-weaning intestinal alterations (26). The impact of dietary fibre supplementation is contrasted and may depend on the type of fibre. Danish investigations reported a reduction in intestinal coliform populations (3) while Australian researchers noted an increased colibacillosis (27) post-weaning. As mentioned above, association of carbohydrates with different fermentability properties are thought to be protective to the gut of young pigs (13-16).

Supplying protein in either a native or a partially hydrolysed form did not make any difference (26). By contrast, various nitrogenous compounds have proven to be of interest. Supplementing diets with glycine and alanine was earlier shown to reduce post-weaning diarrhoea through the stimulation of the production of the so-called anti-secretory factor (28). Glutamine, glutamate and arginine were also shown to have positive effects on growth and intestinal tissue alterations (29, 30).

Thus, various dietary solutions are possible for improving gut health status post-weaning in pigs. However, further research is warranted to determine the optimal doses and possible associations between compounds.

Conclusion and perspectives

Numerous factors interact with developmental patterns for generating spatial-temporal changes in the intestinal physiology, microbiology and immunology of the pig, often leading to transient disorders at weaning. Recent data has allowed the definition of a first phase of acute responses including intestinal hyper-secretion and increased tight junction permeability together with cell protection system (HSP) and pro-inflammatory cytokine gene over-expression. This is followed by a chronic phase of restoration of intestinal architecture and functions leading to maturation towards more adult-type profiles. Weaning also appears to favour a transient instability and reduced diversity of the intestinal microflora. Its stabilisation could be stimulated by subtle manipulation of dietary carbohydrate fermentability. Recent data highlighted the fact that some, but not all, dietary factors could contribute to alleviate post-weaning intestinal disorders.

The cross-talk between the intestinal epithelium, the microflora and the local immune system are still not clearly understood and, therefore, awaits further investigation. Efforts should also be aimed at elucidating the modes of actions of in-feed antibiotic alternatives with known positive properties and at developing new solutions, more natural, and acceptable by the consumers.

Acknowledgements

Many studies reported here were financially supported by the European Union (HEALTHYPIGUT contract n° QLK5-CT2000-00522) to whom we are grateful. The authors thank all the contributors to this research programme.

References

(1) Pluske JR *et al*, 1997. Livest Prod Sci, 51:215-236.
(2) Akkermans ADL *et al*, 2003. Proc 9th Int Symp on Digestive Physiology in Pigs, Ball RO (Ed), Banff, Canada. Vol.1, 49-56.
(3) Jensen BB *et al*, 2003. Proc 9th Int Symp on Digestive Physiology in Pigs, Ball RO (Ed), Banff, Canada. Vol.1, 103-119.
(4) Stokes CR *et al*, 2000. Proc 8th Int Symp on Digestive Physiology in Pigs, Lindberg & Ogle (Eds), Uppsala, Sweden, 59-65.
(5) McCracken BA *et al*. 1999. J Nutr 129:613-619.
(6) David JC *et al*, 2002. J Nutr 132:2551-2561.
(7) Spreeuwenberg MAM *et al*, 2001. J Nutr 131:1520-1527.
(8) Boudry G *et al*., 2004. J Nutr, 134: in press.
(9) Savage DC, 1977. Annu Rev Microbiol, 31:107-133.
(10) Zoetendal EG *et al*, 1998. Appl Env Microbiol, 64:3854-3859.
(11) Klüss J *et al*, 2003. Arch Anim Breeding 46 (Spec. Iss.):101-106.
(12) Leser TD *et al*, 2001. Appl Env Microbiol 68:673-690.
(13) Konstantinov SR *et al*, 2003. FEMS Microbiol Ecol, 43:225-235.
(14) Konstantinov SR *et al*, 2004. Appl Environ Microbiol, 70: in press.
(15) Konstantinov SR *et al*, 2004. Anim Res : in press.
(16) Williams BA *et al*, 2001. Nutr Res Rev 14:207-227.
(17) Stokes CR *et al*, 2004. Anim Res, in press.
(18) Rothkötter HJ *et al*, 1991. Pediatr Res 29:237-242.
(19) Miller BG *et al*, 1994. Br J Nutr 71:615-625.
(20) Miller BG *et al*, 1984. Am J Vet Res 45:1730-1733.
(21) Pié S *et al*, 2004. J Nutr 134:641-647.
(22) Brooks PH *et al*, 2001. In: "The weaner pig: nutrition and management", Varley MA & Wiseman J (Eds), CAB Int, Wallingford, 153-178.
(23) Van Dijk AJ *et al*, 2001. Livest Prod Sci, 68:263-274.
(24) Jiang R *et al*, 2000. J Nutr, 130:21-26.
(25) Touchette KJ *et al*, 2002. J Anim Sci, 80:494-501.
(26) Spreeuwenberg MAM, 2002. PhD dissertation, University of Wageningen, The Netherlands.
(27) McDonald DE *et al*, 2001. Br J Nutr 86:487-498.
(28) Goransson L, 1997. In "Recent advances in animal nutrition", Garnsworthy PC & Wiseman J (Eds), Nottingham University Press, Nottingham, 45-56.
(29) Wu G *et al*, 1996. J Nutr 126:2578-2584.
(30) Ewtushick AL *et al* 2000. Can J Anim Sci 80:653-662.