Review Article

Fate of undigested proteins in the pig large intestine: What impact on the colon epithelium?

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ABSTRACT

Apart from its obvious agronomic interest in feeding billions of people worldwide, the porcine species represents an irreplaceable experimental model for intestinal physiologists and nutritionists. In this review, we give an overview on the fate of proteins that are not fully digested in the pig small intestine, and thus are transferred into the large intestine. In the large intestine, dietary and endogenous proteins are converted to peptides and amino acids (AA) by the action of bacterial proteases and peptidases. AA, which cannot, except in the neonatal period, be absorbed to any significant level by the colonocytes, are used by the intestinal microbes for protein synthesis and for the production of numerous metabolites. Of note, the production of the AA-derived metabolites greatly depends on the amount of undigested polysaccharides in the pig's diet. The effects of these AA-derived bacterial metabolites on the pig colonic epithelium have not yet been largely studied. However, the available data, performed on colonic mucosa, isolated colonic crypts and colonocytes, indicate that some of them, like ammonia, butyrate, acetate, hydrogen sulfide (H_2S), and p-cresol are active either directly or indirectly on energy metabolism in colonic epithelial cells. Further studies in that area will certainly gain from the utilization of the pig colonic organoid model, which allows for disposal of functional epithelial unities. Such studies will contribute to a better understanding of the potential causal links between diet-induced changes in the luminal concentrations of these AA-derived bacterial metabolites and effects on the colon epithelial barrier function and water/electrolyte absorption.

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1. Introduction

One major role of the large intestine is the absorption of water and electrolytes by the absorptive colonocytes (Brown and O'Grady, 1997). These differentiated cells present in the colonic epithelium are derived from a small number of stem cells located at the bottom of the colonic crypts (Van der Flier and Clevers, 2009). The fully-mature colonocytes are finally exfoliated in the large intestine luminal content by a process referred to as apoptosis (Yen and Wright, 2006). The colonic epithelium thus represents a dynamic structure that constitutes the border between the luminal content and the "milieu intérieur" (inner medium, as defined by the French physiologist Claude Bernard in 1855). When compared to the situation in the small intestine, the large intestine luminal fluid is characterized by a much longer transit time, and accordingly by a bacterial population much more abundant than the one found in the small intestine content, especially when considering the proximal part of the small intestine (Schippa and Conte, 2014; Dining, 2016). The transit time in a 65-kg pig colon is rather variable among individuals, ranging from 23 to 56 h (Le Gall et al., 2009). The rapidly growing bacterial population utilizes both endogenous and dietary compounds that have not been (or not fully) digested in the small intestine (Beaumont and Blachier, 2020) for its own metabolism.

A large part of bacteria present in the intestine are excreted in the fecal material, while a minor part of these bacteria (the so-

called adherent bacteria) remains in close proximity with the intestinal epithelial cells (Yin et al., 2011). The colonic epithelium is thus facing a complex mixture of bacteria and numerous compounds of both endogenous and dietary origin, together with bacterial metabolites resulting from the microbiota metabolic activity (Blachier et al., 2007). However, the colonic epithelium and the luminal content are not directly in contact since a specialized population of differentiated epithelial cells, namely the mucous-secreting goblet cells, allows the formation of 2 layers of mucus that exert a protective role for the colonic epithelium from deleterious compounds (Johansson et al., 2013). Interestingly, the composition of colonic mucins in pigs is dependent on age and dietary characteristics (Turck et al., 1993).

In that overall context, the aim of the present manuscript is to give an overview on what is known in the pig model regarding the fate of undigested proteins in the pig large intestine, and the production of metabolites derived from amino acids (AA) by the bacteria of the large intestine microbiota. Finally the effects of AA-derived metabolites on the colonic epithelial cell metabolism and physiology, notably regarding mitochondrial ATP production, will be presented.

2. The pig model for the study of the colonic ecosystem and intestinal physiology

Apart from its obvious agronomic interest in feed, billions of people worldwide, the pig represents an irreplaceable experimental model for intestinal physiologists and nutritionists, as well as gastroenterologists (Yin et al., 2017). The pig model is generally a more relevant model for extrapolation to humans than rodents (Chalvon-Demersay et al., 2017). Pigs are truly omnivorous, make spontaneous individual meals, and display similarities with humans regarding nutritional requirements (Patterson et al., 2008; Mudd and Dilger, 2008). Interestingly, regarding the large intestine, in newborn pigs fed with colostrum, the mucosal weight is markedly increased within few days (Wang and Xu, 1996), indicating rapid intestinal development after birth in the porcine species.

The pig model is advantageous since it allows the recovery of a much larger number of colonic epithelial cells than rodent models, notably in neonates and suckling animals, thus allowing for testing of the effects of dietary intervention on cell metabolism and physiology at different stages of development (Blachier et al., 1993). In addition, the size of newborn and suckling piglets allows tissue samplings, and it is also feasible to practice in pig multi-catheterization and blood sampling without marked anemia, even in kinetics experiments with several time-points (Blachier et al., 1999). However, the use of the pig model for research purposes requires extensive areas for breeding, and is a source of abundant polluting substances in biological fluids, a situation that makes the use of the pig model in urban areas difficult to consider. However, the use of the mini pig model represents an important alternative for research use, when considering these latter drawbacks.

Despite the numerous advantages in the utilization of the pig model, when comparing pigs and humans, differences are measured in the gut anatomy (Kararli, 1995) and microbiota composition. Xiao and collaborators have determined the gut microbiota characteristics in pig fecal samples (Xiao et al., 2016) and found in pigs a total of 7.7 million non-redundant genes representing 719 metagenomic species. When comparing the functional pathways identified in pig and humans, 96% of the functional pathways in humans are present in the catalogue of the pig microbiota community, suggesting that the pig represents a good model for extrapolation of pig fecal microbiota data to humans. However, it is noticeable that conversely only 78% of the pathways

found in the pig gut metagenome are present in humans, raising the view that the specific functionality of gut microbiota may be greater in pigs than in humans (Wang et al., 2020b).

3. Large intestine microbiota: composition, diversity and metabolic activity

The large intestine microbiota include bacteria, archaea, viruses, fungi and protozoa (Aluthge et al., 2019). Considering the whole ecosystem in the large intestine, and in order to better understand how the microbiota can influence the host physiology and metabolism, it is important to consider both the composition of the microbiota and the microbial diversity. At birth, a piglet is suddenly plunged into a complex bacterial environment including the bacteria of the maternal vagina and sow's feces and rearing environment. The knowledge of the changes in bacterial composition in the intestinal content according to different parameters, including gestational stages in sows (Kong et al., 2016; Ji et al., 2019), intra-uterine growth status (Xiong et al., 2020), mode of piglet delivery (Wang et al., 2013), early dietary conditions (Poulsen et al., 2017), age of animals and weaning time (Konstantinov et al., 2003; Inoue et al., 2005), has represented important milestones. However, one of the challenges for future research is to better understand how different bacterial compounds and products of bacterial metabolic activity represent important components for the crosstalk between the host and its microbiota.

Regarding the bacterial compounds that are recognized as signaling by the host, including for instance the much studied bacterial lipopolysaccharide (LPS) as a compound of the Gram-negative bacteria, readers are referred to recent excellent reviews on that topic (Saad et al., 2016; Gomes et al., 2018; Fuke et al., 2019), since this aspect will not be developed in the present paper.

Regarding the products of bacterial activity in the large intestine luminal fluid, studies in mammals have focused on the short-chain fatty acid production, and notably on butyrate production from undigestible carbohydrates (Tiwari et al., 2019). More recent studies have studied the impact of AA-derived bacterial metabolites on the intestinal epithelial cell metabolism and physiology as presented in the present review.

4. Transfer of proteins from the small intestine to the large intestine in pigs, bacterial metabolite production, and consequences

4.1. Transfer of luminal proteins through the ileo-caecal junction

Although the process of protein and peptide digestion from both dietary and endogenous origin is an efficient process in mammals in general, and in pigs in particular, a significant portion of undigested (or not fully digested) protein and other N-containing substances enter the caecum through the ileo-caecal junction. The amount of N-containing substances entering the large intestine is dependent on several parameters including the amount and source of dietary proteins, as well as the pig developmental stage (Gilbert et al., 2018; Le Gall et al., 2007). For instance, in piglets, a decreased feed intake that is associated with changes in the small intestine epithelium morphology is often observed at weaning. These changes include a notably decreased villus height and increased crypt depth (Nabuurs et al., 1993; Pié et al., 2004). Accordingly, brush-border enzymatic activities and macronutrient final digestion by enterocytes are reduced (Pié et al., 2004). Then, an increased amount of proteins may reach the piglet large intestine after weaning (Gilbert et al., 2018). Such an increased transfer of protein may also happen in low birth weight newborn pigs which are

routinely fed a high-protein diet to speed up their growth rate (Boudry et al., 2014).

This increased transfer can be further amplified when the amount of protein in the diet is increased and/or when less digestible proteins are used in the pig's diet. This is reflected by increased concentrations in the large intestine of several AA-derived bacterial metabolites in the large intestine (Blachier et al., 2019b). Proteins and peptides are degraded in the large intestine by the bacterial proteases and peptidases which release peptides and AA. As a matter of fact, the large digestive capacity of the pig caecum-colon towards proteins has been demonstrated (Just et al., 1981). Recently, supplementation with *Bacillus subtilis* in piglets was found to increase the digestibility of proteins, and this effect was associated with increased growth performance (Lewton et al., 2021). The action of the bacterial proteases presumably plays a major role in the increased protein digestibility observed after *B. subtilis* supplementation (Tang et al., 2019).

Absorption of AA, either from the AA released from undigested proteins or from anabolic activity of intestinal microbiota, is considered to be very low in the mammalian large intestine when compared to the absorptive capacity of the small intestine (Van der Wielen et al., 2017). In pigs, absorption of AA in the colon has been demonstrated only in newborn piglets (Smith and James, 1976), but this capacity diminishes rapidly within few days after birth (James and Smith, 1976; Sepulveda and Smith, 1979), and has been shown to be at most very limited in growing pigs (Just et al., 1981).
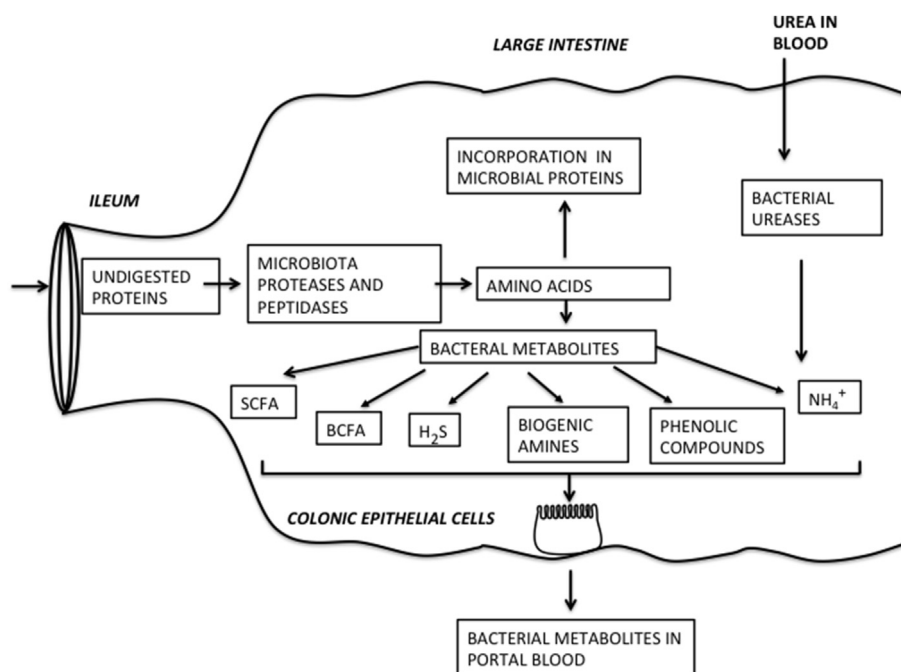
4.2. Production of bacterial metabolites from the amino acids depending on the diet

The AA can be used by the microbial population for their own protein synthesis and for catabolism giving rise to numerous metabolic end-products, the so-called bacterial metabolites that include biogenic amines, phenols, indoles, hydrogen sulfide, ammonia, short-chain fatty acids, and branched-chain fatty acids (Portune et al., 2016) (Fig. 1). Among these metabolites, some are not exclusively produced through protein fermentation. For instance, the short-chain fatty acids acetate, propionate, and butyrate that can be produced by the large intestine microbiota from the AA alanine, aspartate, glutamate, glycine, lysine, and threonine (Blachier et al., 2007), can generally be produced in much greater amounts from the undigestible carbohydrates present in standard diets (Tiwari et al., 2019). In addition, organic acids, like succinate, lactate, oxaloacetate and formate can all be formed from protein-derived AA (Blachier et al., 2007). In contrast, some AA, namely valine, leucine, and isoleucine, are specific precursors for branched-chain fatty acids (namely isobutyrate, isovalerate and 2-methyl-butyrate), and thus are considered as indicators of protein fermentation in the large intestine (Yao et al., 2016).

Branched-chain fatty acid production can be modified by some specific dietary compounds in pigs. Supplementation with fermented soybean meal, characterized notably by higher oligosaccharide content in unfermented form, reduces isovalerate in the piglet large intestine (Zhang et al., 2018). In addition, by feeding piglets with a low protein diet supplemented with essential AA, branched-chain fatty acids are also decreased when compared with a normal protein diet (Luo et al., 2015). Dietary supplementation with chitooligosaccharide or soybean oligosaccharide in mini-piglets reduces the isobutyrate, and isovalerate concentrations in the colon (Kong et al., 2014; Zhou et al., 2014). These results demonstrate the important role played by undigestible carbohydrates in the protein fermentation process in the pig large intestine.

It is feasible to examine the global effects of modifications of the amount and/or of the source of dietary protein on the large intestine epithelium in terms of parameters like water absorption and electrolyte absorption/secretion, epithelial barrier function, and colonic epithelium renewal. For instance, Richter and collaborators have shown that a higher amount of highly fermentable crude protein in the pig colon reduces the expression of the tight junction protein claudin-1, -2, and -3, an effect associated with a

Fig. 1. Production of bacterial metabolites from amino acids in the large intestine. This schematic presentation shows the transfer of undigested dietary and endogenous proteins from the small intestine to the large intestine, and the production of bacterial metabolites from amino acids. Several among these metabolites have been shown to act on the epithelial colonic cell metabolism and physiology, before being released in the portal vein. SCFA = short-chain fatty acids; BCFA = branched-chain fatty acids; H_2S: hydrogen sulfide; NH_4^+ = ammonium.

modification of the transcytotic movement across the colonic epithelium (Richter et al., 2014). In order to illuminate the "black box" between changes in the characteristics of the pig diet and biochemical/functional consequences at the colonic level, it is necessary to document firstly the impact of dietary changes on the bacterial metabolite concentrations on the colonic epithelial cells (Blachier et al., 2017).

Regarding ammonia (considered as the sum of NH_4^+ and NH_3) concentration, it has been found that this compound is increased by a higher amount of dietary proteins in the weaned pig distal colon (Pieper et al., 2014). Ammonia is produced by the intestinal microbiota from AA deamination (Smith and Macfarlane, 1997) and by the hydrolysis of urea by the bacterial ureases (Moran and Jackson, 1990) (Fig. 1). Ammonia concentration measured in the distal colon of pigs fed with a high-protein diet is in the 12.1 to 21.0 mmol/L range (Pieper et al., 2014; Bikker et al., 2006). By reducing crude protein in the pig diet or replacing a part of the dietary crude protein by essential AA in pig food, ammonia is decreased in the colon and caecum (Peng et al., 2017; Luo et al., 2015; Htoo et al., 2007; Zhang et al., 2016). In addition, a high-protein/low fiber diet increases caecal ammonia concentration in piglets (Stumpff et al., 2013). By supplementing mini-piglets with chitooligosaccharide or soybean oligosaccharide, it is possible to decrease the ammonia concentration in the colon (Kong et al., 2014; Zhou et al., 2014). Supplementation of pigs fed a western-type diet with wheat arabinoxylan, used as soluble fiber, decreased ammonia concentration in the large intestine (Williams et al., 2016). Interestingly, zinc oxide, used at pharmacological doses to prevent diarrhea in pigs, decreases notably ammonium fecal concentration (Janczyk et al., 2015). These latter results are of interest notably when considering that 1) a high-protein diet that increases ammonia concentration in the colon decreases fecal consistence due to higher water content (Pieper et al., 2012; Wellock et al., 2006), 2) ammonium chloride at 20 mmol/L concentration decreased the monocarboxylate transporter 1 (MCT1) gene expression in pig colon (Villodre Tudela et al., 2015), this transporter allowing butyrate uptake in colonocytes, and 3) supplementation with sodium butyrate in weaned piglets decreased the incidence of diarrhea (Huang et al., 2015). It is thus tempting to propose from these latter data that an increased ammonia concentration in the large intestine, following high-protein diet consumption, would favor the diarrheal process by interfering with the process of butyrate uptake by the colonic absorptive cells and thus with the action of butyrate on sodium/water absorption by the colonic epithelium (Bedford and Gong, 2018).

Concerning the concentration of indole and indolic compound (that is produced by the intestinal microbiota from tryptophan), and concentrations of phenolic compounds, that include phenol, p-cresol, and phenyl-containing compounds (that are produced by the fermentation of L-tyrosine), they are increased in the colon of weaned pigs fed a high-protein diet (Pieper et al., 2014). In piglets, 88% of the excreted p-cresol is recovered in the urine, while 12% is recovered in feces (Yokoyama et al., 1982), thus indicating that a large proportion of p-cresol, continuously produced by the intestinal microbiota, is absorbed from the luminal content to the bloodstream before excretion in urine, the remaining being kept in the large intestine content before periodic fecal excretion. Lower crude dietary protein intake reduces phenol and indole concentrations in the pig caecum and colon (Zhang et al., 2016; Zhang et al., 2017). Supplementation with lignocellulose decreases phenols and indoles in the piglet large intestine (Pieper et al., 2014). Supplementation of pigs fed a westernized diet containing cooked red meat with arabinoxylan-rich fraction from wheat diminishes p-cresol concentration in the caecum, and phenol concentration in

the colon (Belobrajdic et al., 2012). These latter studies confirm that undigestible carbohydrate consumption decreases protein fermentation in the pig large intestine.

Regarding the biogenic amine production, the concentrations of the polyamines putrescine and spermidine are higher in the colon of piglets fed a high-protein diet (Pieper et al., 2012), as well as the concentration of histamine (Pieper et al., 2012) that is produced from histidine fermentation. Conversely, reducing crude protein content in the pig diet results in a decrease of the caecal putrescine concentration (Htoo et al., 2007). Other biogenic amines including tyramine and cadaverine are decreased by feeding pigs with diets containing lower crude protein content (Zhang et al., 2016). In addition, moderate protein restriction in finishing pigs decreases putrescine, histamine, and spermidine in the colonic content (Fan et al., 2017). By feeding piglets with a low protein diet supplemented with essential AA, cadaverine concentration in the caecum was found to be decreased when compared to normal protein diet (Luo et al., 2015). Dietary proline supplementation in pregnant mini-pigs increases the concentrations of the bioamine 1,7-heptyl diamine and phenylethylamine in the proximal colon content (Ji et al., 2018), suggesting increased AA catabolism by the intestinal microbiota after such supplementation. L-proline supplementation in sows during pregnancy markedly increases the concentration of putrescine and spermidine in the whole large intestine (containing the luminal fluid) in fetuses when compared to the isonitrogenous control group of sows (Wang et al., 2020a).

Thus, overall, feeding pigs with high-protein diet is associated, as expected, with increased concentration of AA-derived bacterial metabolites in the large intestine, while undigestible carbohydrates generally decrease protein fermentation in the pig large intestine. Dietary starch types are apparently an important parameter in determining their effect on protein fermentation in the pig large intestine since for instance, pea starch decreases the concentrations of putrescine, cadaverine, skatole, indole and phenol in the colon when compared with tapioca starch (Yu et al., 2019). Interestingly, a recent study performed with suckling and weaned piglets, has shown that the relative fecal concentrations of the bacterial metabolites methylamine, dimethylamine, cadaverine, succinate, and 3-(4-hydroxyphenylpropionate) are higher during the suckling period than after weaning. In contrast, the relative concentrations of acetate and propionate are higher after weaning than during the suckling period (Beaumont et al., 2021), thus indicating modification of the luminal environment in the distal part of the pig digestive tract during the suckling to weaning transition.

4.3. Effects of amino acid-derived bacterial metabolites on colonic metabolism and physiology in pigs

4.3.1. Colonic epithelial cell energy metabolism

Colonic epithelial cells are characterized by a high energy demand since the colonic epithelium is entirely renewed within a few days, thus requiring intense anabolic metabolism and high rate of ATP synthesis. In addition, absorbing colonocytes, because of high sodium (and water) absorption, require energy for the functioning of the Na/K ATPase (Blachier et al., 2009). Pig colonocytes can use several substrates for energy production including mainly short-chain fatty acids, glutamine, and to a lesser extent glucose used both in the oxidative and in the glycolytic pathways (Darcy-Vrillon et al., 1993) (Fig. 2). Regarding the availability of these different energy substrates, it is important to consider that absorptive colonocytes, as polarized cells, can obtain their fuels from both the luminal and the basolateral sides. Ammonium chloride at a 10 mmol/L concentration, thus in the range of ammonium concentrations found in the colonic content of piglets receiving a high-
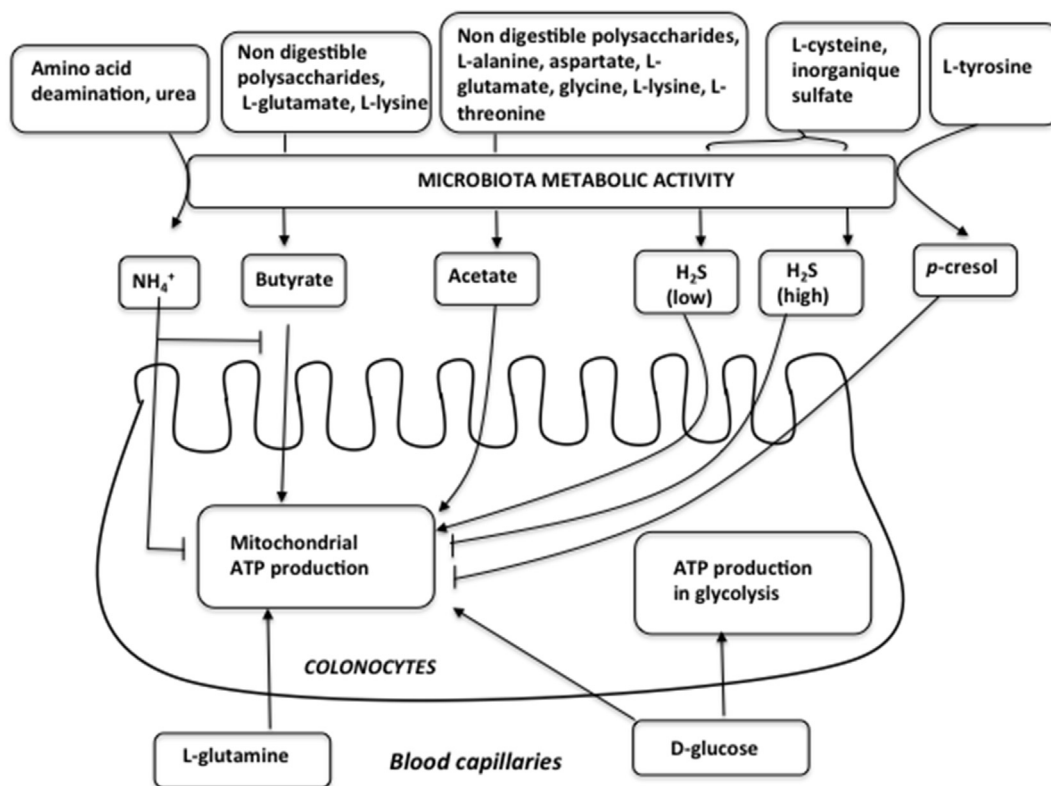
Fig. 2. Energy production in colonocytes from the utilization of fuels originating from blood and luminal fluid. This schematic presentation shows the substrates used by the colonocytes for ATP production, as well as the inhibitory effect of ammonium, hydrogen sulfide (H_2S), and p-cresol on mitochondrial ATP production when present in excess.

protein diet, markedly reduces butyrate but not acetate oxidation in pig colonocytes (Darcy-Vrillon et al., 1996), suggesting that ammonia affects butyrate metabolism at the steps of activation and/or beta-oxidation (Fig. 2). In this study, ammonia was found to increase glucose utilization in the glycolysis, indicating that ammonia modifies substrate utilization in colonocytes. However, when considering the varied ATP production rates for substrate utilization in the mitochondrial oxidative pathways in comparison to glycolysis, it can be predicted that increased glycolysis will not be able to compensate for decreased butyrate oxidation in terms of ATP production. However, ammonium, when tested at 50 mmol/L concentration does not affect pig colonic crypt cell viability after 4 h incubation (Leschelle et al., 2002), maybe because of an inhibitory effect of ammonium on colonic epithelial cell proliferation (Mouillé et al., 2003), and consequently with a decreased requirement of ATP for anabolic processes. Such an inhibition of the ATP requirement would avoid a sharp decrease of the ATP intracellular concentration in colonocytes that may affect their viability.

Of note, from experiments in conscious pigs receiving increasing doses of ammonium chloride in the colonic lumen, and by measuring ammonia in the portal and arterial blood, it has been determined that the colonic epithelium of 50 kg pigs has the capacity to absorb up to 4 g of ammonia after intra-colic acute injection, and this, without saturation of the hepatic ureagenesis capacity (Eklou-Lawson et al., 2009). Further works are required to test if an increase of ammonia concentration in the large intestine following high-protein consumption may induce an energy-deficient state in the colonic epithelium that would affect the pig colonic epithelium renewal and/or functions.

Hydrogen sulfide (H_2S) is produced by the intestinal microbiota from different dietary and endogenous S-containing substrates including cysteine and sulfomucins (Blachier et al., 2010). At low

micromolar concentrations, H_2S is a mineral energy substrate for colonic absorptive cells, while at low millimolar concentrations, H_2S is a metabolic trouble maker as it inhibits the cytochrome oxidase activity in the mitochondrial respiratory chain (Blachier et al., 2019a), and thus reduces mitochondrial ATP production (Fig. 2). In the pig large intestine, the net production of H_2S by the intestinal microbiota increased from the caecum towards the distal colon (Poulsen et al., 2012). However, at a concentration of 1 mmol/L, the hydrogen sulfide donor NaHS does not affect pig colonic crypt cell viability after 4 h incubation (Leschelle et al., 2002) maybe because of compensating processes in colonic epithelial cells as observed in in vitro experiments (Leschelle et al., 2005).

Excessive p-cresol has been shown to decrease mitochondrial oxygen consumption (Andriamihaja et al., 2015), and the ATP intracellular content in a colonic epithelial cell line (Wong et al., 2016), indicating that p-cresol affects energy metabolism in colonocytes (Fig. 2). In vivo experiments in animal model are required to examine to what extent such alteration may affect the colonic epithelium physiology.

4.3.2. Colonic epithelium renewal and barrier function

Few data are available regarding the effects of AA-derived bacterial metabolites on colonic epithelial renewal and barrier function.

Polyamines that include putrescine, spermidine and spermine are well known to be involved in intestinal epithelium renewal in mammals (Timmons et al., 2012). In colonic epithelial cells, intracellular polyamines may originate from the luminal side, presumably mainly from the metabolic activity of the microbiota (Blachier et al., 2011), and to a lesser extent from polyamine release in the luminal content after exfoliation of mature colonocytes. Polyamines from dietary supply that have not been absorbed by the

small intestine (Bardocz, 1993) represent an additional source for colonic epithelial cells, together with the intracellular synthesis from L-ornithine (Blachier et al., 2011). Spermidine and spermine are measurable in the colostrum and milk recovered from sows (Motyl et al., 1995), and spermidine increases progressively from week 1 to 7 of lactation (Kelly et al., 1991). However, the possible effect of polyamines on the rapid growth of the colonic mucosa in piglets after birth remains to be examined.

Indolic compounds derived from tryptophan have been reported to increase intestinal epithelial cell tight junction resistance (Bansal et al., 2010; Venkatesh et al., 2014; Shimada et al., 2013), while regulating intestinal homeostasis during aging (Powell et al., 2020). However, indole-derived indoxyl sulfate is well known to act as a uremic toxin, and an excessive amount of this latter compound is deleterious for the tubular kidney cells (Liu et al., 2018; Cheng et al., 2020), raising the view that indolic compounds represent a double-edged sword. To the best of our knowledge, there are no available data regarding the ratio of the beneficial over deleterious effects of an increased concentration of indole in the piglet large intestine (notably after the consumption of a high-protein diet), notably on the colon and kidney physiology.

p-cresol decreases the transepithelial resistance and increases the paracellular transport in colonocyte monolayers (Wong et al., 2016). These results suggest that p-cresol may alter the colonic epithelial barrier function, but the in vivo consequences of an increased p-cresol concentration in the colonic luminal concentration remains here again to be determined.

4.3.3. Water and electrolyte absorption/secretion by the colonic epithelium

There is still little information on the effects of AA-derived bacterial metabolites on water and electrolyte movement through the colonic epithelium.

Histidine-derived histamine induces luminal chloride secretion as measured in Ussing chamber experiments using pig colonic mucosa (Ahrens et al., 2006). Interestingly, colonic tissues recovered from piglets fed a high-protein diet display increased activity of histamine-degrading enzymes like diamine oxidase and histamine N-methyltransferase, thus suggesting metabolic adaptive processes towards increased luminal histamine production (Kröger et al., 2013; Aschenbach et al., 2009). Putrescine supplementation in the diet of weaning piglets decreased the diarrhea index, and thus the water content in the intestinal luminal fluid, in association with an increased butyrate concentration in the colon (Liu et al., 2019).

5. Conclusions and perspectives

From the available data obtained in pigs, there is no doubt that the composition of the food given to the animals, notably in terms of quantity and source of dietary protein, plays a major role on the concentration of numerous amino acid-derived bacterial metabolites recovered in the large intestinal fluid. Importantly, the amount and nature of the undigestible carbohydrates (that include fibers) in the diet are also important parameters in modulating the concentration of these metabolites. Since these metabolites are produced from specific amino acids (Fig. 1), the amino acid composition of dietary proteins, in addition to protein digestibility in the small intestine will fix, for a given microbiota composition, the luminal concentrations of these metabolites, and thus the luminal environment of the colonic epithelium.

However, it is worth noting at this step of discussion that the luminal environment is not only depending on the bacterial metabolite concentrations, but on other parameters including the

pH and osmolarity, which may also impact the colonic epithelium physiology. It is worth noting regarding this latter point that bacterial metabolite concentration and pH/osmolarity are not disconnected. For instance, when the pH in the large intestine is more acidic, the concentration of the diffusible active compound H_2S increases, while the concentration of hydrosulfide anion decreases (Blachier et al., 2017). In the same line of thinking, a more acidic luminal pH will decrease the proportion of the anionic form of butyrate, resulting in a lower uptake of this compound through the monocarboxylate transporter isoform 1 by colonocytes. Lastly, for ammonia, a lower pH in the colon will displace the equilibrium between NH_3 and NH_4^+, favoring the proportion of NH_4^+, which, in contrast with NH_3 (which easily penetrates cell membranes), needs a specific transporter to enter colonocytes (Handlogten et al., 2005). Regarding osmolarity, the production of bacterial metabolites vs. their absorption rate through the colonic epithelium may lead to their accumulation in the luminal fluid with a resultant increased osmolarity.

Changes in the osmolarity of the extracellular medium are known to affect *in vitro* colonocyte metabolism and functions (Grauso et al., 2019). Also, changes in the luminal pH may affect *per se* the colonic epithelial cell physiology (Blachier et al., 2017). In order to document the consequences of changes of the luminal environment of the pig colonic epithelium, in terms of beneficial or deleterious effects, further experimental works are obviously required focusing on the effects of bacterial metabolites, tested either individually or in combination at different concentrations, on the colonic epithelial cells. For that aim, it is possible to implant a canula in the pig colon in order to inject endoluminally bacterial metabolites of interest (Eklou-Lawson et al., 2009) and then to recover colonic mucosal biopsies for analysis. It is also feasible to test the cytotoxic effect of a mixture of luminal compounds, by performing the so-called "fecal water cytotoxicity test". In that test, after nutritional intervention, mixtures of water-soluble compounds are recovered from the fecal material, and are then tested for their global cytotoxic effect on colonic epithelial cells (Beaumont et al., 2017). Regarding the effects of individual amino acid-derived bacterial metabolites on colonic epithelial cells, except for ammonia which has been the subject of several studies in the pig model, few works has been devoted to the effects of these compounds on parameters like sodium and water absorption, epithelial renewal, mucus secretion and associated barrier function in association with the effects on the energy metabolism in colonic epithelial cells. This is indeed an important research objective as loss of the colonic epithelial homeostasis may contribute to colonic mucosa inflammation (Garcia-Hernandez et al., 2017) and diarrhea (Thiagarajah et al., 2018).

Most of the results obtained in this area are derived from experiments using either colonic biopsies, colonic crypts or isolated colonic absorptive cells that can survive only for a limited time. New experimental models have been developed more recently, notably the use of organoids isolated from pig colon which represents a model closer to the in vivo situation (Sharbati et al., 2015; Callesen et al., 2017). This model offers the possibility to replicate the metabolism and functions of the different epithelial cell phenotypes in the course of colonic epithelial cell proliferation, differentiation and apoptosis. Also, the use of organoids maintained in culture (or kept at very low temperature for further culture) allows us to diminish the number of animals used for experiments. Such a model will be much useful for further tests of bacterial metabolites for their effects, either beneficial or deleterious, on colonic epithelium renewal and function. In the future, from such experimental works, we can hope to define optimal dietary conditions, notably in terms of the amount and sources of

protein and undigestible carbohydrates, for an optimal luminal environment, that will help maintain a healthy state in the colonic epithelium.

Author contributions

Francois Blachier writes the original draft that was reviewed and edited by **Xiang-Feng Kong** and **Mireille Andriamihaja**.

Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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