Amino Acids (2017) 49:2099–2106
DOI 10.1007/s00726-017-2497-z

REVIEW ARTICLE

Pig models on intestinal development and therapeutics

Lanmei Yin[1] · Huansheng Yang[1,2] · Jianzhong Li[1] · Yali Li[1] · Xueqing Ding[1] · Guoyao Wu[2,3] · Yulong Yin[1,2]

Received: 20 July 2017 / Accepted: 23 September 2017 / Published online: 6 October 2017

Abstract The gastrointestinal tract plays a vital role in nutrient supply, digestion, and absorption, and has a crucial impact on the entire organism. Much attention is being paid to utilize animal models to study the pathogenesis of gastrointestinal diseases in response to intestinal development and health. The piglet has a body size similar to that of the human and is an omnivorous animal with comparable anatomy, nutritional requirements, and digestive and associated inflammatory processes, and displays similarities to the human intestinal microbial ecosystem, which make piglets more appropriate as an animal model for human than other non-primate animals. Therefore, the objective of this review is to summarize key attributes of the piglet model with which to study human intestinal development and intestinal health through probing into the etiology of several gastrointestinal diseases, thus providing a theoretical and hopefully practical, basis for further studies on mammalian nutrition, health, and disease, and therapeutics. Given the comparable nutritional requirements and strikingly similar brain developmental patterns between young piglets and humans, the piglet has been used as an important translational model for studying neurodevelopmental outcomes influenced by pediatric nutrition. Because of similarities in anatomy and physiology between pigs and mankind, more emphasises are put on how to use the piglet model for human organ transplantation research.

Keywords Gastrointestinal tract · Microflora · Pig models · Human gastrointestinal diseases · Pediatric nutrition

Handling Editors: C.-A. A. Hu, Y. Yin, Y. Hou, G. Wu, Y. Teng.

✉ Huansheng Yang
yhs@hunnu.edu.cn

✉ Yulong Yin
yinyulong@isa.ac.cn

[1] Animal Nutrition and Human Health Laboratory, School of Life Sciences, Hunan Normal University, Changsha City 410081, Hunan, China

[2] Chinese Academy of Science, Institute of Subtropical Agriculture, Research Center for Healthy Breeding of Livestock and Poultry, Hunan Engineering and Research Center of Animal and Poultry Science and Key Laboratory for Agroecological Processes in Subtropical Region Scientific Observation and Experimental Station of Animal Nutrition and Feed Science in South-Central, Ministry of Agriculture, Changsha 410125, Hunan, China

[3] Texas A&M University, College Station, TX 77843, USA

Introduction

It is well established that the gastrointestinal (GI) tract is responsible for the first physiological step of absorbing nutrients into the entire body's cells and has a critical impact on the regulation of the growth and development of young mammals (Guilloteau et al. 2010). However, a range of factors can affect gastrointestinal health and result in all kinds of gastrointestinal diseases, such as inflammatory bowel disease (IBDs) (Pouillart et al. 2010), necrotizing enterocolitis (NEC) (Jiang and Sangild 2014), and short bowel syndrome (SBS) (Jiang and Sangild 2014; Gonzalez et al. 2015) and intrauterine growth restriction (IUGR) (Jiang and Sangild 2014; D'Inca et al. 2011). These diseases not only cause great damage to the human health, but also dampen the economic returns of the whole husbandry. Thus, animal models can be used to study the pathogenesis and mechanism of the GI development and diseases. Rodent animals are most frequently utilized because of their low cost in breeding, feeding, and handling (Heinritz et al. 2013). However, when

studying intestinal development and diet–microbiota–host cell interactions, the differences in intestinal morphology, microbiota, and other distinct physiological states between rodents and humans cannot be ignored (Corpet and Pierre 2005). Another animal model is the primate, although primates are comparable to humans and were reported to be an excellent model for study, the shortcomings of them are rather expensive, and the most important is the ethical issues, namely animal welfare (Flamm 2013). The physiological and anatomical similarity between humans and pigs makes the pig more preferable than other non-primate models for the study of intestinal development and diseases (Sciascia et al. 2016). Moreover, the piglet is a human-sized omnivorous animal with similar nutritional requirements and the intestinal microbial ecosystem to those in humans (Heinritz et al. 2013). In addition, the pig has been used as a model to assess microbiota–host cell interactions, and pigs show similar IBD pathogenesis and pathophysiology to humans (Heinritz et al. 2013; Duncan et al. 2008). Finally, the intestinal metabolism of amino acids (such as arginine, glutamine, glutamate, and proline) is similar between the pig and human (Wu 1998; Wu et al. 2004, 2016). The focus of this review is to outline the similarities between pigs and other animal models, highlight the suitability and significance of the pig model in studying human GI diseases, and provide the scientific basis for the further research on the intestinal development and health.

Advances in using the pig as a model for human GI

There is an increasingly number of scientists using animal models as a tool to study the mechanism of several diseases with the intention of improving both animal and human health. The previous studies have been conducted on the murine animals to advance our understanding of human hematopoiesis, innate and adaptive immunity, autoimmunity, infectious diseases, cancer biology, and regenerative medicine, which have not only provided some constructive suggestions for the subsequent researchers, but also revealed a variety of disadvantages in this model (Shultz et al. 2007). These models cannot reflect the mechanism of pathogenesis and immunity faithfully because of the difference from human condition. The similarities between pigs and human, including anatomy, physiology, biochemistry, genetics, and even pathology (Panepinto and Phillips 1986; Parsons and Wells 1986; Rispat et al. 1993), make the porcine an optimal model to study various microbial infection diseases (Meurens et al. 2012). Moreover, because of the bacterial resistance and alterations of intestinal flora caused by abuse of antibiotics, a growing body of probiotics and prebiotics have been used as the alternative feed additives to improve gut health and microbiota (Adhikari and Kim 2017; Sánchez et al. 2017). The swine has been frequently used as an animal model to study the mechanism of intestinal diseases related to altered intestinal microbes. Nowadays, obesity has become a troublesome problem that puzzles people either in developed countries or in developing countries. Since the pig has the advantage of low genetic variance, homogeneous feeding manner, and the absence of individual living habits (such as smoking and alcohol drinking), and the similar pathological response to high in energy intakes between pigs and humans, the previous studies have used the genome as a tool for conducting comparative studies to understand the pathogenesis of high incidence of obesity to support the use of pig model for identifying genes and their variants associated with energy storage defects through the activation of both hormonal and biochemical pathways (Brambilla and Cantafora 2004; Tan et al. 2012). As is sketched out in Table 1, comparing with the rodent and primate animal models, the pig has been employed as a primary model for human gastrointestinal studies.

Table 1 Comparisons among pig, rodent, and primate animals

Animal models	Description	References
Pigs		
Advantages	Similar anatomy, physiology, nutritional requirements, microbiota diversity, and digestive and metabolic process.	Labib et al. (2004), Patterson et al. (2008), Gandarillas and Bas (2009)
Disadvantages	Different intestinal weight and length.	
Rodents		
Advantages	Low costs in breeding, feeding, and handling.	Heinritz et al. (2013)
Disadvantages	Great nutritional, physiological, and metabolic differences.	
Primates		
Advantages	Similar diets to the human.	Flamm (2013), Oosterloo et al. (2014)
Disadvantages	Moral and ethical issues, extremely expensive.	

Pig as a model for human GI development

With the further advancement of animal nutrition research, many studies have been carried out in the mammalian and rodent animals at the metabolic level (Patterson et al. 2008). Rodent models, murine animals in particular, offer several advantages over other species. However, a few studies, if any, have been done in the swine in the GIT as the target organ. What is known to us all is that the GIT plays a vital role in the nutrient supply, digestion, and absorption, and has a crucial impact on the entire organism, while gut microbes are of great significance in human health (Quigley 2017), which leads to many studies with swine. The diet of weaning piglets is shifted from high-fat, low-carbohydrate milk to a high-carbohydrate and low-fat feed (Yang et al. 2016). What is worse, when combined with the changes in the social and physiological environments, these contribute to the alteration of the intestinal flora, while the gut microflora in animals speeds up gut maturity and stimulates a robust immune response (Butler et al. 2002; Haverson et al. 2007; Scharek et al. 2005). As a consequence, several intestinal bacterial populations have been related to comprehensive health conditions, even with conditions not directly connected with the GIT such as diabetes (Qin et al. 2012), asthma (Azad and Kozyrskyj 2012), arthritis and pregnancy (Koren et al. 2012), and disorders of the immune system (Wen et al. 2008; Brown et al. 2012). In the same way, the GIT of human is easily influenced by the changes in the weather and surroundings. On the other hand, rodent models are hampered by various physiological differences between murine and primate animals (Rothkötter and Summerfield 2009). Moreover, a majority of enteric diseases seen in human cannot show clinical manifestations typically in mice and rats (Jeong et al. 2010). In addition, individuals are genetically diverse and vulnerable to be affected by many environmental factors and accessible to consume different diets, all of these factors that have different effects on the intestinal ecology (Zhang et al. 2013).

Many animal models, including mammals (rats, man, guinea-pigs, rabbits, pigs, sheep, mice, dogs, cows, and cats) and non-mammalian vertebrates (chickens and frogs) have been used to study GIT development (Guilloteau et al. 2010). In the case of the GIT, the animal model whose GIT function and pattern of development are most similar to man should be considered (Guilloteau et al. 2010). There are extensive structural and functional changes in intestinal epithelium in utero, but the stages of intestinal maturation are markedly different in various mammalian species at birth, and these variations are closely dependent on the duration of the gestational period (Guilloteau et al. 2010; Yang et al. 2013). Rodent animals, which are born after a short gestation, have relatively immature GIT at birth and do not achieve independence until after weaning. In these species, adult diets are poorly tolerated until relatively late in postnatal life, and adult-type GIT functions develop rapidly after weaning. In contrast, intestinal development in precocial animals, which have a long gestation period, such as pig and sheep, occurs early in utero (Pacha 2000; Guilloteau et al. 2010). The major developmental events in GIT of these animals take place both before and after birth. The porcine GIT development occurs during in fetal and neonatal periods like humans and other primates (Pacha 2000; Guilloteau et al. 2010). From the perspective of a GIT, utilizing the 'sow–piglet' dyad as a model of the human 'mother–infant' dyad seems reasonable when studying the effects of nutritional programming on human GIT development, this knowledge will also lead to nutritional recommendations and therapies for prevention and treatment after validation in human subjects (Guilloteau et al. 2010). As mentioned above, swine as a model is fundamental for the research of human GIT development.

Pig as a model for pediatric nutrition

It is well known that pediatric nutrition plays a critical role in the growth and development of the whole organism. There is a growing body of evidence that high rates of neonatal morbidity and mortality pose a great threat to both medical and husbandry (Odle et al. 2014). At birth, the newborn mammal goes through a great transition from a sterile intrauterine environment with a sustained nutrient supply from maternal placenta, to an adequate microbe environment with intermittent intake of maternal milk or infant formula through the GIT (Sangild et al. 2013). In addition, after birth, the newborn mammal gets nutrients from maternal milk maintaining the further growth and development. However, an increasing and constant nutritional requirement cannot satisfy the demands of neonates. Fortunately, the popularity of infant formula may provide a substitute for the neonates. Owing to an ever increasing number of substances added to the infant formula, the safety and efficacy of these substances are the major concern, which prompt researchers to determine if an appropriate neonatal animal model could be found for alleviating this phenomena and testing the toxicity (Swindle et al. 2012). The physiological similarity between neonatal pigs and human infants in terms of digestive and associated metabolic processes places neonatal pigs in a superior position over other non-primate models for the study of pediatric nutrition and metabolism (Heinritz et al. 2013). Furthermore, neonatal pigs have similar nutritional requirements and are anatomically similar to newborns, while nutrients regulate GIT function and health (Sciascia et al. 2016). When it comes to comparing drugs with substances new to infant, several aspects should be taken into account. First, the similarities and differences in

biology and physiology of the animal model should be taken into consideration for the administered substances (Forster et al. 2010). Second, the mode of administration and matrix should be analogical to the natural diet of a human infant to the greatest extent (Flamm 2013). In addition, the chosen animal models should imitate the stage of development of the intended target (Swindle et al. 2012), namely a neonate or an infant. Finally, it is of great importance to set the same parameters as for drugs when assessing new substances to infant formula (Swindle et al. 2012), especially the overall growth. The previous studies have demonstrated that due to their very immature development of the GIT (Guilloteau et al. 2009), it is extremely difficult to feed neonatal rodents through oral administration (Flamm 2013). Consequently, rodent animals are not fit for testing new substances in infant formula. In contrast, although neonatal monkeys can utilize a high-fat diet (Flamm 2013), a concern is that primates are expensive and their use in research is beset with severe ethical issues (Odle et al. 2014; Flamm 2013), many researchers are unpleased to make use of them. From the nutrition and metabolism point of view, the piglet model has uncovered mechanistic underpinnings of nutrient function at molecular and cellular levels and proven effective for whole-animal preclinical safety screening prior to direct studies with infants (Odle et al. 2014). The previous studies aim at the preterm pig and prove that it is a highly translational large animal model for ameliorating parenteral and enteral feeding regimens for preterm infants, with some findings relevant also for the clinical care of term newborn infants and pigs. Meanwhile, the preterm pig model stands for a collaborative research platform for basic biology, pediatric medicine, and agricultural and veterinary science (Sangild et al. 2013). To sum up, the neonatal pigs appear to be the most optimal neonatal model for testing the safety and efficacy of new substances in infant formula, and the piglets represent an adaptable and beneficial model for pediatric nutrition.

Pig as a model for human gastrointestinal diseases

The homeostasis of intestinal microbial ecosystem is of primary importance to stay health for animals and humans (Quigley 2017). An alteration of intestinal microflora contributes to a multitude of GI diseases. In recent years, the prevalence of high-throughput sequencing or "next-generation" sequencing technology, including 16s RNA and metagenomics (Maropola et al. 2015; Yang et al. 2014; Zhang et al. 2013), greatly helps analyzing the microbial diversity and a certain intestinal microbe population, elucidating the mechanism of specific intestinal diseases, and developing therapies for preventing or curing them. Amplicons of 16S rRNA V6 region were deep-sequenced to monitor the extent to which the transplanted human microbiomes are changed in the pig. Extracting DNA from fecal and analyzing colonic microbiomes stemming from the same animal indicate that feces closely replicate the colonic microbiome, which suggests that the pig intestine can be colonized with human fecal microbiomes to generate a realistic model of the human GIT (Zhang et al. 2013). Utilizing the pig model to explore the interaction between microbiome and host in healthy and diseased animals will enable further studies carried out on how to cure or alleviate disease of the GI tract. In addition, swine share a similar gastrointestinal anatomy and physiology with humans and, therefore, may represent a more suitable animal model for the study of gastrointestinal disease therapeutics (Liu et al. 2017).

Inflammatory bowel disease

Inflammatory bowel disease (IBD), a chronic inflammation of the GIT, is characterized by a dysfunction of the mucosal immune system and resistance of activated T cells to apoptosis (Ahern et al. 2010) and is composed of Crohn's Disease (CD) and ulcerative colitis (UC) (Randhawa et al. 2014) with an expanding worldwide incidence and prevalence (Xavier and Podolsky 2007). While CD and UC are associated with alterations of the microbiota (Joossens et al. 2011; Kostic et al. 2014). Among the various models, trinitrobenzene sulfonic acid (Pouillart et al. 2010) and dextran sodium sulphate induced Crohn's disease and ulcerative colitis (Kim et al. 2010) respectively, these colitis models are extensively used in the pig. The sign of these diseases is crypt absences, ulceration, increased lamina propria thickness, and inflammation (O'Connor et al. 2009). In addition, acetic acid has been administered to the colon of pigs to induce intestinal injury (Hou et al. 2014). Inflammation with inflammatory cells (such as neutrophilic granulocyte and macrophages) leads to the deregulation of intestinal immune responses and a great weight loss. Although the exact pathogenesis remains poorly understood, mounting evidence showed that inflammation involves a multiple of interactions between the immune system, genetic susceptibility, and the environment, most importantly is the gut microbiota (Campieri and Gionchetti 2001). That humans and pigs are genetically similar and are vulnerable to be impacted by the external environment (Yang et al. 2014). Furthermore, the known physiological and anatomical similarities between pigs and humans place the pig in a superior position to other more common animal models for studying IBD (Dedhia et al. 2016). Because physiological differences between humans and rodents are significant (Wang and Donovan 2015), the use of rodent models alone has undoubtedly hindered progress and complicated the translation of biomedical research findings into effective preventive or intervention therapies for IBD.

Necrotizing enterocolitis and short bowel syndrome

The NEC refers to necrosis of the complete mucosa, and occurs mainly in the distal small intestine, namely the ileum, and colon of infants and piglets, whose incidence is greater in infants born more premature at earlier gestational ages and associated high morbidity and mortality (Heinritz et al. 2013). Of note, deficiencies of arginine and glutamine occur in preterm infants 1 week before the onset of NEC (Becker et al. 2000). Several studies have shown that the constant and frequent morbidities are linked to both the site and the length of intestinal resection. Neonates, suffering from severe NEC, may be subject to intestinal resection and result in SBS. Anatomical or functional loss of a marked length of the small intestine, which is characterized by a condition with significant malabsorption and "intestinal failure", contributes to an incapability of avoiding loss of intestinal fluid, absorbing nutrient, and maintaining energy balance (O'Keefe et al. 2006; Aunsholt et al. 2014). Faced with the great challenges, researchers need to consider whether an animal model can be used to study the etiology of NEC and SBS, maintain intestinal health, and guarantee livestock production. Anatomical and physiological similarities between infants and neonatal pigs make the newborn piglets be preferable to find therapies for intestinal disorder diseases. Neonatal piglets are vulnerable to be affected by a range of factors such as poor nutritional conditions and variable microbe environment (Jacobi and Odle 2012), while intestinal infections and digestive syndromes are key causes of neonatal pig morbidity (Ask et al. 2012). Meanwhile, gut immaturity, along with general bacterial colonization, is the crucial factors that give rise to clinical NEC (Oosterloo et al. 2014). Compared with the infant, the superiority of the preterm piglet model animal to study symptoms of NEC, coupled with a similar development of the symptoms, arises from the very parallel clinical and histological characteristics of this syndrome (Bjornvad et al. 2008). An essential approach to understanding the etiology and underlying biology of NEC is the use of in vivo experimental animal models, especially neonatal pigs. More recently, pigs have emerged as an animal model of NEC and utilized to identify the role of bacterial colonization, prematurity, parenteral nutrition, and antibiotic therapy (Oosterloo et al. 2014). Furthermore, SBS is connected to the extensive resection of the intestine among preterm infants; thus, preterm pig models could be utilized for intestinal resection and SBS studies (Sangild et al. 2009; Vegge et al. 2013). Using pig as an animal model, unknown pathways and new prognostic disease markers for SBS were identified by proteomic analysis (Jiang and Sangild 2014). For example, the finding that citrulline is synthesized de novo in pigs exclusively from glutamine, glutamate, and proline in enterocytes led to the use of serum citrulline as a biomarker for intestinal function in preterm infants with short bowel syndrome (Wu and Morris 1998; Rhoads et al. 2005). Intestinal HSPs, iron metabolism proteins, and proteins related to amino acid (e.g., arginine) and glucose metabolism are consistently affected by NEC progression, and some of these proteins are also impacted by SBS (Jiang and Sangild 2014). Explorative non-targeted proteomics provides ideas about the cellular pathways involved in intestinal adaptation during the critical neonatal period. Proteomics, coupled with other bioinformatics techniques, contributes to get a more comprehensive understanding of the intestinal adaptation during NEC and SBS (Jiang and Sangild 2014).

Intrauterine growth restriction

The IUGR, refers to the slowed growth (growth means an increase in the number and size of cells or in the mass of tissues) and the impaired development (development, in other words, changes in the structure and function of cells or tissues) of the mammalian embryo/fetus or its organs during gestation period (Wu et al. 2006), remains a significant issue in human health and livestock production. Low birth weight, which resulted from IUGR, is one of the most important causes of neonatal loss. The IUGR negatively affects the whole organism growth performance, and causes damage to the life-long health (Wu et al. 2006). The reasons accounting for the etiology and mechanism of IUGR are as follows: First, the fetal genome plays a vital role in the potential growth in utero (Jansson 2016). However, increasing evidence shows that the intrauterine environment has an indispensable influence on the fetal growth (Dimasuay et al. 2016). Moreover, in mammals, the fetus obtains nutrients from the maternal blood through the placenta, which is the sole source of fetal growth and development in utero. Instead, undernutrition or overnutrition will lead to growth restriction (Wu et al. 2006). Based on the causes of the IUGR, studies utilizing animal models of IUGR will supply the necessary scientific basis to the development of management practices to enhance the efficiency of human health and livestock production. The previous studies compared organ weights and intestinal structure, function and microbiota between control and IUGR newborn piglets that were delivered by cesarean section at full term or prematurely (91% gestation) during the period 0–5 days of age, and demonstrated that IUGR affects intestinal development regardless of gestational age at delivery (D'Inca et al. 2011). Studies in the pig model also indicated that IUGR reduced the density of the intestine at birth and the growth of GIT, which may, therefore, result in slow postnatal growth of IUGR neonates (Xu et al. 1994; Wang et al. 2005). Finally, the pig model can be used to study epigenetic regulation of intestinal development and health in humans (Ji et al. 2016; Li et al. 2017).

Conclusions and perspectives

The pig has been utilized in scientific research as an animal model for humans on intestinal development and health, due to similarities in anatomical structure, physiological characteristics, nutritional requirements, and microbiota diversity. The pig is an appropriate model to study human GI and new knowledge gained from the pig studies can be useful to prevent and cure human GI diseases, such as IBD, NEC, SBS, and IUGR. Moreover, pig model has exerted dramatic effect on the pediatric nutrition, including testing new substances added to the infant formula to ensure its safety to infants' growth. Although considerable progress has been made through the pig model on intestinal development and health, these theoretical studies are in the very early stage far behind production practice, further research is warranted to make efficient use of pigs in biomedical research. We anticipate that with the joint efforts of scientists and livestock workers, results from basic and applied research with pigs will enhance the efficiency of livestock production and improve the wellbeing of humans.

Acknowledgements This work was supported by the National Natural Science Foundation of China (No. 31402089, 31330075), Natural Science Foundation of Hunan Province (2017JJ1020), Huxiang Youth talent Support Program (2016RS3028), Young Elite Scientists Sponsorship Program by CAST (YESS20160086), and Scientific Research Foundation of Hunan Provincial Education Department (17B164).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors. Hence, no informed consent was required for any part of this review.

References

Adhikari PA, Kim WK (2017) Overview of prebiotics and probiotics: focus on performance, gut health and immunity—a review. Ann Anim Sci (in press). doi:10.1515/aoas-2016-0092

Ahern PP, Schiering C, Buonocore S, McGeachy MJ, Cua DJ, Maloy KJ, Powrie F (2010) Interleukin-23 drives intestinal inflammation through direct activity on T cells. Immunity 33:279–288. doi:10.1016/j.immuni.2010.08.010

Ask B, Dahl J, Nielsen MB, Moustsen V (2012) Neonatal piglet traits of importance for survival in crates and indoor pens. J Anim Sci 89:1207–1218. doi:10.2527/jas.2010-3248

Aunsholt L, Jeppesen PB, Lund P, Sangild PT, Ifaoui IB, Qvist N, Husby S (2014) Bovine colostrum to children with short bowel syndrome: a randomized, double-blind, crossover pilot study. Jpn Parenter Enter 38:99–106. doi:10.1177/0148607112469630

Azad MB, Kozyrskyj AL (2012) Perinatal programming of asthma: the role of gut microbiota. Clin Dev Immunol 2012:932072–932081. doi:10.1155/2012/932072

Becker RM, Wu G, Galanko JA, Chen W, Maynor AR, Bose CL, Rhoads JM (2000) Reduced serum amino acid concentrations in infants with necrotizing enterocolitis. J Pediatr 137:785–793

Bjornvad CR, Thymann T, Deutz NE, Burrin DG, Jensen SK, Jensen BB, Mølbak L, Boye M, Larsson LI, Schmidt M (2008) Enteral feeding induces diet-dependent mucosal dysfunction, bacterial proliferation, and necrotizing enterocolitis in preterm pigs on parenteral nutrition. Am J Physiol Gastrointest Liver Physiol 295:G1092–G1103. doi:10.1152/ajpgi.00414.2007

Brambilla G, Cantafora A (2004) Metabolic and cardiovascular disorders in highly inbred lines for intensive pig farming: how animal welfare evaluation could improve the basic knowledge of human obesity. Ann Ist Super Sanità 40:241–244

Brown K, Decoffe D, Molcan E, Gibson DL et al (2012) Diet-induced dysbiosis of the intestinal microbiota and the effects on immunity and disease. Nutrients 4:1095–1119. doi:10.3390/nu4081095

Butler JE, Weber P, Sinkora M, Baker D, Schoenherr A, Mayer B, Francis D (2002) Antibody repertoire development in fetal and neonatal piglets. VIII. Colonization is required for newborn piglets to make serum antibodies to T-dependent and type 2 T-independent antigens. J Immunol 169:6822–6830. doi:10.4049/jimmunol.169.12.6822

Campieri M, Gionchetti P (2001) Bacteria as the cause of ulcerative colitis. Gut 48:132–135. doi:10.1136/gut.48.1.132

Corpet DE, Pierre F (2005) How good are rodent models of carcinogenesis in predicting efficacy in humans? A systematic review and meta-analysis of colon chemoprevention in rats, mice and men. Eur J Cancer 41:1911–1922. doi:10.1016/j.ejca.2005.06.006

Dedhia PH, Bertaux-Skeirik N, Zavros Y, Spence JR (2016) Organoid models of human gastrointestinal development and disease. Gastroenterology 150:1098–1112. doi:10.1053/j.gastro.2015.12.042

Dimasuay KG, Boeuf P, Powell TL, Jansson T (2016) Placental responses to changes in the maternal environment determine fetal growth. Front Physiol 7:12. doi:10.3389/fphys.2016.00012

D'Inca R, Gras-Le Guen C, Che L, Sangild PT, Le Huerou-Luron I (2011) Intrauterine growth restriction delays feeding-induced gut adaptation in term newborn pigs. Neonatology 99:208–216. doi:10.1159/000314919

Duncan SH, Lobley GE, Holtrop G, Ince J, Johnstone AM, Louis P, Flint HJ (2008) Human colonic microbiota associated with diet, obesity and weight loss. Int J Obesity 32:1720–1724. doi:10.1038/ijo.2008.155

Flamm EG (2013) Neonatal animal testing paradigms and their suitability for testing infant formula. Toxicol Mech Method 23:57–67. doi:10.3109/15376516.2012.725108

Forster R, Bode G, Ellegaard L, Jw VDL (2010) The RETHINK project–minipigs as models for the toxicity testing of new medicines and chemicals: an impact assessment. J Pharmacol Tox Met 62:158–159. doi:10.1016/j.vascn.2010.05.003

Gandarillas M, Bas F (2009) The domestic pig (*Sus scrofa domestica*) as a model for evaluating nutritional and metabolic consequences of bariatric surgery practiced on morbid obese humans. Inv Agr 36:163–176. doi:10.4067/S0718-16202009000200002

Gonzalez LM, Moeser AJ, Blikslager AT (2015) Porcine models of digestive disease: the future of large animal translational research. Transl Res 166:12–27. doi:10.1016/j.trsl.2015.01.004

Guilloteau P, Zabielski R, Hammon HM, Metges CC (2009) Adverse effects of nutritional programming during prenatal and early postnatal life, some aspects of regulation and potential prevention and treatments. J Physiol Pharmacol 60(Suppl 3):17–35

Guilloteau P, Zabielski R, Hammon HM, Metges CC (2010) Nutritional programming of gastrointestinal tract development. Is the

pig a good model for man? Nutr Res Rev 23:4–22. doi:10.1017/S0954422410000077
Haverson K, Rehakova Z, Sinkora J (2007) Immune development in jejunal mucosa after colonization with selected commensal gut bacteria: a study in germ-free pigs. Vet Immunol Immunop 119:243–253. doi:10.1016/j.vetimm.2007.05.022
Heinritz SN, Mosenthin R, Weiss E (2013) Use of pigs as a potential model for research into dietary modulation of the human gut microbiota. Nutr Res Rev 26:191–209. doi:10.1017/S0954422413000152
Hou YQ, Wang L, Yi D, Ding BY, Chen X, Wang QJ, Zhu HL, Liu YL, Yin YL, Gong J, Wu G (2014) Dietary supplementation with tributyrin alleviates intestinal injury in piglets challenged with intrarectal administration of acetic acid. Br J Nutr 111:1748–1758
Jacobi SK, Odle J (2012) Nutritional factors influencing intestinal health of the neonate. Nutr Res Rev 26:191–209. doi:10.1017/S0954422413000152
Jansson T (2016) Placenta plays a critical role in maternal-fetal resource allocation. P Natl Acad Sci USA 113:11066–11068. doi:10.1073/pnas.1613437113
Jeong KI, Zhang Q, Nunnari J, Tzipori S (2010) A piglet model of acute gastroenteritis induced by Shigella dysenteriae Type 1. J Infect Dis 201:903–911. doi:10.1086/650995
Ji Y, Wu ZL, Dai ZL, Wang XL, Li J, Wang BG, Wu G (2016) Fetal and neonatal programming of postnatal growth and feed efficiency in swine. J Anim Sci Biotechnol 8:42
Jiang P, Sangild PT (2014) Intestinal proteomics in pig models of necrotising enterocolitis, short bowel syndrome and intrauterine growth restriction. Proteom Clin Appl 8:700–714. doi:10.1002/prca.201300097
Joossens M, Huys G, Cnockaert M, Preter VD, Verbeke K, Rutgeerts P, Vandamme P, Vermeire S (2011) Dysbiosis of the faecal microbiota in patients with Crohn's disease and their unaffected relatives. Gut 60:631–637. doi:10.1136/gut.2010.223263
Kim CJ, Kovacs-Nolan JA, Yang C, Archbold T, Fan MZ, Mine Y (2010) l-Tryptophan exhibits therapeutic function in a porcine model of dextran sodium sulfate (DSS)-induced colitis. J Nutr Biochem 21:468–475. doi:10.1016/j.jnutbio.2009.01.019
Koren O, Goodrich JK, Cullender TC, Spor A, Laitinen K, Bäckhed HK, Gonzalez A, Werner JJ, Angenent LT, Knight R (2012) Host remodeling of the gut microbiome and metabolic changes during pregnancy. Cell 150:470–480. doi:10.1016/j.cell.2012.07.008
Kostic AD, Xavier RJ, Gevers D (2014) The microbiome in inflammatory bowel disease: current status and the future ahead. Gastroenterology 146:1489–1499. doi:10.1053/j.gastro.2014.02.009
Labib S, Erb A, Kraus M, Wickert T, Richling E (2004) The pig caecum model: a suitable tool to study the intestinal metabolism of flavonoids. Mol Nutr Food Res 48:326–332. doi:10.1002/mnfr.200400022
Li N, Wang W, Wu G, Wang J (2017) Nutritional support for low birth weight infants: insights from animal studies. Br J Nutr 117:1390–1402
Liu Y, Wang X, Hou Y, Yin Y, Qiu Y, Wu G, Hu C-AA (2017) Roles of amino acids in preventing and treating intestinal diseases: recent studies with pig models. Amino Acids 49:1277–1291. doi:10.1007/s00726-017-2450-1
Maropola MK, Ramond JB, Trindade M (2015) Impact of metagenomic DNA extraction procedures on the identifiable endophytic bacterial diversity in Sorghum bicolor (L. Moench). J Microbiol Meth 112:104–117. doi:10.1016/j.mimet.2015.03.012
Meurens F, Summerfield A, Nauwynck H, Saif L, Gerdts V (2012) The pig: a model for human infectious diseases. Trends Microbiol 20:50–57. doi:10.1016/j.tim.2011.11.002
O'Connor W Jr, Kamanaka M, Booth CJ, Town T, Nakae S, Iwakura Y, Kolls JK, Flavell RA (2009) A protective function for interleukin 17A in T cell-mediated intestinal inflammation. Nat Immunol 10:603–609. doi:10.1038/ni.1736
Odle J, Lin X, Jacobi SK, Kim SW, Stahl CH (2014) The suckling piglet as an agrimedical model for the study of pediatric nutrition and metabolism. Annu Rev Anim Biosci 2:419–444. doi:10.1146/annurev-animal-022513-114158
O'Keefe SJ, Buchman AL, Fishbein TM, Jeejeebhoy KN, Jeppesen PB, Shaffer J (2006) Short bowel syndrome and intestinal failure: consensus definitions and overview. Clin Gastroenterol H 4:6–10. doi:10.1016/j.cgh.2005.10.002
Oosterloo BC, Premkumar M, Stoll B, Olutoye O, Thymann T, Sangild PT, Burrin DG (2014) Dual purpose use of preterm piglets as a model of pediatric GI disease. Vet Immunol Immunop 159:156–165. doi:10.1016/j.vetimm.2014.02.012
Pacha JIRI (2000) Development of intestinal transport function in mammals. Physiol Rev 80:1633–1667
Panepinto LM, Phillips RW (1986) The Yucatan miniature pig: characterization and utilization in biomedical research. Lab Anim Sci 36(4):344–347
Parsons AH, Wells RE (1986) Serum biochemistry of healthy Yucatan miniature pigs. Lab Anim Sci 36:344–347
Patterson JK, Lei XG, Miller DD (2008) The pig as an experimental model for elucidating the mechanisms governing dietary influence on mineral absorption. Exp Biol Med 233:651–664. doi:10.3181/0709-MR-262
Pouillart PR, Dépeint F, Abdelnour A, Msc LD, Vincent O, PhD J-CMM, Msc JYM, PhD DCM, Younes H, Bsc DW (2010) Nutriose, a prebiotic low-digestible carbohydrate, stimulates gut mucosal immunity and prevents TNBS-induced colitis in piglets. Inflamm Bowel Dis 16(5):783–794
Qin J, Li Y, Cai Z, Li S, Zhu J, Zhang F, Liang S, Zhang W, Guan Y, Shen D (2012) A metagenome-wide association study of gut microbiota in type 2 diabetes. Nature 490:55–60. doi:10.1038/nature11450
Quigley EMM (2017) Gut microbiome as a clinical tool in gastrointestinal disease management: are we there yet? Nat Rev Gastro Hepat 14:315–320. doi:10.1038/nrgastro.2017.29
Randhawa PK, Singh K, Singh N, Jaggi AS (2014) A review on chemical-induced inflammatory bowel disease models in rodents. Korean J Physiol Pharmacol 18:279–288. doi:10.4196/kjpp.2014.18.4.279
Rhoads JM, Plunkett E, Galanko J, Lichtman S, Taylor L, Maynor A, Weiner T, Freeman K, Guarisco JL, Wu G (2005) Serum citrulline correlates with enteral tolerance and bowel length in infants with short bowel syndrome. J Pediatr 146:542–547
Rispat G, Slaoui M, Weber D, Salemink P, Berthoux C, Shrivastava R (1993) Haematological and plasma biochemical values for healthy Yucatan micropigs. Lab Anim UK 27:368–373
Rothkötter HJ, Summerfield A (2009) Anatomical particularities of the porcine immune system—a physician's view. Dev Comp Immunol 33:267–272. doi:10.1016/j.dci.2008.06.016
Sánchez B, Delgado S, Blanco-Míguez A, Lourenço A, Gueimonde M, Margolles A (2017) Probiotics, gut microbiota and their influence on host health and disease. Mol Nutr Food Res (in press). doi:10.1002/mnfr.201600240
Sangild PT, Thymann T, Boving LA, Støy AC, Lund P, Jeppesen PB, Sigalet D, Qvist N (2009) Prematurity reduces functional adaptation to intestinal resection in piglets. Clin Nutr Supp 4:156–157. doi:10.1016/S1744-1161(09)70363-1
Sangild PT, Thymann T, Schmidt M, Stoll B, Burrin DG, Buddington RK (2013) Invited review: the preterm pig as a model in pediatric gastroenterology. J Anim Sci 91:4713–4729. doi:10.2527/jas2013-6359
Scharek L, Guth J, Reiter K, Weyrauch KD, Taras D, Schwerk P, Schierack P, Schmidt MF, Wieler LH, Tedin K (2005) Influence of a probiotic Enterococcus faecium strain on development of

the immune system of sows and piglets. Vet Immunol Immunop 105:151–161. doi:10.1016/j.vetimm.2004.12.022
Sciascia Q, Daş G, Metges CC (2016) Review: the pig as a model for humans: Effects of nutritional factors on intestinal function and health. J Anim Sci 94:441–452. doi:10.2527/jas2015-9788
Shultz LD, Ishikawa F, Greiner DL (2007) Humanized mice in translational biomedical research. Nat Rev Immunol 7:118–130. doi:10.1038/nri2017
Swindle MM, Makin A, Herron AJ Jr, Frazier KS (2012) Swine as models in biomedical research and toxicology testing. Vet Pathol 49:344–356. doi:10.1177/0300985811402846
Tan BE, Li XG, Yin YL, Wu ZL, Liu C, Tekwe CD, Wu G (2012) Regulatory roles for L-arginine in reducing white adipose tissue. Front Biosci 17:2237–2246
Vegge A, Thymann T, Stoll B, Lund P, Qvist N, Hartmann B, Jelsing J, Jeppesen PB, Holst JJ, Sangild PT (2013) Glucagon-like Peptide2 induces rapid adaptation and improves function of the remnant intestine following intestinal resection in preterm neonates. Gastroenterology 140:S197–S198. doi:10.1016/S0016-5085(11)60798-1
Wang M, Donovan SM (2015) Human microbiota-associated swine: current progress and future opportunities. ILAR J 56:63–73. doi:10.1093/ilar/ilv006
Wang T, Huo YJ, Shi F, Xu RJ, Hutz RJ (2005) Effects of intrauterine growth retardation on development of the gastrointestinal tract in neonatal pigs. Biol Neonate 88:66–72
Wen L, Ley RE, Volchkov PY, Stranges PB, Avanesyan L, Stonebraker AC, Hu C, Wong FS, Szot GL, Bluestone JA (2008) Innate immunity and intestinal microbiota in the development of Type 1 diabetes. Nature 455:1109–1113. doi:10.1038/nature07336
Wu G (1998) Intestinal mucosal amino acid catabolism. J Nutr 128:1249–1252
Wu G, Morris SM Jr (1998) Arginine metabolism: nitric oxide and beyond. Biochem J 336:1–17
Wu G, Jaeger LA, Bazer FW, Rhoads JM (2004) Arginine deficiency in premature infants: biochemical mechanisms and nutritional implications. J Nutr Biochem 15:442–451
Wu G, Bazer FW, Wallace JM, Spencer TE (2006) Board-invited review: intrauterine growth retardation: implications for the animal sciences. J Anim Sci 84:2316–2337. doi:10.2527/jas.2006-156
Wu ZL, Hou YQ, Hu SD, Bazer FW, Meininger CJ, McNeal CJ, Wu G (2016) Catabolism and safety of supplemental L-arginine in animals. Amino Acids 48:1541–1552
Xavier RJ, Podolsky DK (2007) Unravelling the pathogenesis of inflammatory bowel disease. Nature 448:427–434. doi:10.1038/nature06005
Xu RJ, Mellor DJ, Birtles MJ, Reynolds GW, Simpson HV (1994) Impact of intrauterine growth retardation on the gastrointestinal tract and the pancreas in newborn pigs. J Pediatr Gastroenterol Nutr 18:231–240
Yang H, Xiong X, Yin Y (2013) Development and Renewal of Intestinal Villi in Pigs. In Nutritional and Physiological Functions of Amino Acids in Pigs (pp 29–47). Springer Vienna
Yang F, Chia N, Mazur M, Pettigrew J, Schook LB, White BA (2014) Genetically identical co-housed pigs as models for dietary studies of gut microbiomes. Microbiome Sci Med 1:45–54. doi:10.2478/micsm-2014-0002
Yang H, Xiong X, Wang X, Li T, Yin Y (2016) Effects of weaning on intestinal crypt epithelial cells in piglets. Sci Rep UK 6:36939. doi:10.1038/srep36939
Zhang Q, Giovanni W, Saul T (2013) A pig model of the human gastrointestinal tract. Gut Microbes 4:193–200. doi:10.4161/gmic.23867