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Review article

Novel and disruptive biological strategies for resolving gut health challenges in monogastric food animal production

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ABSTRACT

Use of feed antibiotics as growth promoters for control of pathogens associated with monogastric food animal morbidity and mortality has contributed to the development of antimicrobial resistance, which has now become a threat to public health on a global scale. Presently, a number of alternative feed additives have been developed and are divided into two major categories, including 1) the ones that are supposed to directly and indirectly control pathogenic bacterial proliferation; and 2) the other ones that are intended to up-regulate host gut mucosal trophic growth, whole body growth performance and active immunity. A thorough review of literature reports reveal that efficacy responses of current alternative feed additives in replacing feed antibiotics to improve performances and gut health are generally inconsistent dependent upon experimental conditions. Current alternative feed additives typically have no direct detoxification effects on endotoxin lipopolysaccharides (LPS) and this is likely the major reason that their effects are limited. It is now understood that pathogenic bacteria mediate their negative effects largely through LPS interactions with toll-like receptor 4, causing immune responses and infectious diseases. Therefore, disruptive biological strategies and a novel and new generation of feed additives need to be developed to replace feed antibiotic growth promoters and to directly and effectively detoxify the endotoxin LPS and improve gut health and performance in monogastric food animals.

1. Introduction

Evidence of the domestication of a handful of animal species, including pigs, can be traced back to early recorded human civilization (Diamond, 2002; Groenen et al., 2012). Animal production plays important roles in the continued human evolution in both physical and mental health as well as social and economic viability (Pond and Lei, 2001; Fan, 2013). Animal production, in providing meats, contributes greatly to social and economic activities and wellbeing (Pond and Lei, 2001; Fan et al., 2008). Current world food animal production is represented by compound feed production at 980 million dry tons valued at about $460 billion US per year with monogastric poultry and swine feeding accounting for 72% of the total production volume (Alltech, 2015). Feed cost typically accounts for about 70% of the operation expenses in intensive food animal production. China's compound feed production is at 183 million dry tons, accounting for about 18.7% of the world, market value, which is estimated at about $170 billion US per year (Alltech, 2015). Current intensive food animal production practices are faced with challenges and obstacles to evolve the industry to be socially, economically and environmentally sustainable with an emerging one being the antimicrobial resistance concern (Levy, 1998; Gorbach, 2001).

For the past several decades, antibiotics have been by far the most cost-effective way to maintain feed efficiency and health status in intensive animal production systems, including poultry and swine (Cromwell, 2001; Looft et al., 2012). However, dietary supplementation of sub-therapeutic levels of feed antibiotics in animal production has now been recognized as the main determinant of antimicrobial resistance (Davies et al., 1999; Forslund et al., 2013). Antibiotic-resistant commensal and pathogenic bacteria can be directly spread to humans via retail meat products (Johnson et al., 2005), transferred into the environment after manure

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application (Chee-Sanford et al., 2009), and are further transmitted into ecosystems (Koike et al., 2007). Antimicrobial resistance that originates from use of feed antibiotics in livestock industries contributes to the development of multidrug resistance "Superbugs" that are jeopardizing effective treatment of infectious diseases in human patients (Gorbach, 2001), pets (Umber and Bender, 2009), and wild birds (Guenther et al., 2010), becoming a major threat to global public health (WHO, 2014).

On the other hand, use of sub-therapeutic levels of feed antibiotics neither eliminates food-borne pathogenic bacteria from food production animals (Casewell et al., 2003; Looft et al., 2012) nor detoxifies lipopolysaccharide (LPS) endotoxin (Koyama et al., 2002; Chen et al., 2010). These food animals' gut systems are a safe hideout and reservoir for the pathogenic bacteria (Johnson et al., 2005; Manges and Johnson, 2012). Thus, alternative strategies need to be developed to replace feed antibiotics and to effectively detoxify pathogenic endotoxins and minimize pathogenic bacterial populations while maintaining and enhancing efficiency and health status of agricultural production food animals including poultry and swine.

2. History of sub-therapeutic levels of antibiotics as growth promoters

Antibiotics are arguably the most successful form of chemotherapy discovered in the history of medicine (Wright, 2007). The initial use of antibiotics as growth promoters in food animal production dates back to the "golden era" of antibiotic discovery during 1940's to 1960's (Visek, 1978; Wright, 2007). The widespread use of sub-therapeutic levels of feed antibiotics primarily in monogastric food animal production has been practiced since the 1960's when the average cost of feed-grade antibiotics was dramatically reduced (Cromwell, 2001). Mode of actions associated with the use of sub-therapeutic levels of feed antibiotics for improving feed efficiency and health status in food animals is still not completely clear. The classic mechanisms of feed antibiotics for improving feed efficiency include the control of bacterial overgrowth, more essential luminal nutrients available for host growth, and stimulation of immunity (Visek, 1978; Cromwell, 2001; Gaskins et al., 2002). Feed antibiotics are recently shown to increase gut microbial genes relating to energy production and conversion (Looft et al., 2012). Thus, applications of sub-therapeutic levels of feed antibiotics are still regarded as the most cost-effective way to improve feed utilization efficiency and health status in monogastric food animal nutrition. An effective alternative strategy needs to be developed in order to replace feed antibiotics as growth promoters in monogastric food animal production.

Use of sub-therapeutic levels of feed antibiotics induces significant changes in the gut microbiota by reducing bacterial diversity (McCracken et al., 2001; Looft et al., 2012). Studies have demonstrated that failure to establish and maintain a normal gut microbiota would promote innate immune activation and may impair immune homeostasis (Mulder et al., 2009, 2011; Schmidt et al., 2011). Thus, young animals fed sub-therapeutic levels of feed antibiotics are susceptible to innate immune activation. Furthermore, feed antibiotics do not eliminate commensal and pathogenic bacteria, and this is largely due to the fact that bacteria develop antibiotic resistance genes (Forslund et al., 2013). For example, feed antibiotics increase commensal and pathogenic *Escherichia coli* populations in weanling pigs' gut (Looft et al., 2012). Animals' gut is a main reservoir of pathogenic bacteria, which is the primary source of food-borne pathogens (Johnson et al., 2005; Manges and Johnson, 2012). Thus, applications of sub-therapeutic levels of feed antibiotics as growth promoters do not disinfect pathogenic bacteria in animal production. There is an urgent need to develop a fundamentally new approach to prevent dysbiosis in swine production and minimize pathogenic bacterial impact on meat safety and public health concerns.

Use of sub-therapeutic levels of feed antibiotics is known to attenuate but does not fundamentally resolve the traditional challenges of post-weaning growth lag or check and the weaning-associated pig morbidity and mortality, which hampers the swine industry (Varley and Wiseman, 2001). Scientifically, this is because feed antibiotics do not completely eliminate intestinal Gram-negative bacteria that produce and shed LPS endotoxin into the gut lumen (Rietschel et al., 1994; Erridge et al., 2002; Kulp and Kuehn, 2010). Biologically, feed antibiotics do not detoxify the LPS endotoxin. Furthermore, intestinal apical alkaline phosphatase, being recognized as the primary endogenous LPS detoxification enzyme, is less effective with its less physiological optimal pH of 10.5 (Fan et al., 1999), as well as its decreased maximal enzyme activity and enzyme affinity or enzyme efficiency in potentially detoxifying the LPS endotoxin in the weanling pig (Lackeyram et al., 2010), making weanling pigs susceptible to the development of endotoxemia.

It has now been demonstrated that LPS is effectively transported across the intestinal epithelial apical membrane via an endocytosis process in conjunction with long-chain fatty acid assembly into chylomicrons and makes its way into the systemic circulation (Ghoshal et al., 2009; Pendyala et al., 2012). Dr. Bruce Beutler was a recipient for the 2011 Nobel Prize in Physiology or Medicine for leading research in revealing that toll-like receptor 4 (TLR4) was the LPS receptor in the endotoxin-mediated inflammation via innate immunity (Poltorak et al., 1998). Luminal and systemic circulating LPS endotoxin triggers gut mucosal local (McCracken et al., 2001; Pié et al., 2004), and systemic inflammation (Klasing, 1988) via innate immunity mediated by TLR4, enhancing gut permeability (Salzman et al., 1994; Lallès et al., 2007), releasing pro-inflammatory cytokines into systemic circulation (Klasing, 1988), resulting in anorexia (Johnson, 1998; Buchanan and Johnson, 2007), and causing sickness behavior and diminishing welfare in animals (Kelley et al., 2003; Dantzer et al., 2008). Hence, intestinal luminal LPS and systemic endotoxemia are largely responsible for monogastric food animal morbidity and mortality. Applications of sub-therapeutic levels of feed antibiotics as growth promoters do not detoxify LPS endotoxin and associated pro-inflammatory cytokine effects in major food production monogastric animals such as poultry and swine.

3. Antimicrobial resistance and conventional alternative strategies

Antimicrobial resistance has now become a major threat to global public health (WHO, 2014). Antibiotic resistance genes existed before the "antibiotic era" (Aminov and Mackie, 2007). All genes encode proteins that show resistance to antimicrobials and are referred to as resistome (Wright, 2007). Development of antimicrobial resistance in swine production has been well documented to occur in two major distinctive types, including antibiotic-induced antibiotic resistance (e.g., Aminov and Mackie, 2007) and heavy metal-specific (i.e., Cu and Zn, in particular) antimicrobial resistance (Fard et al., 2011) due to feeding pharmacological levels of Cu (200 to 250 mg/kg) and Zn (2,000 to 3,000 mg/kg) as growth promoters (Fan, 2013).

To address the antibiotic resistance concerns, European Union banned sub-therapeutic levels of feed antibiotics during 1997 to 1999, which actually led more therapeutic antibiotics uses in food animal production and deteriorated antibiotic resistance to therapeutic antibiotics (Casewell et al., 2003). The Casewell et al.

(2003) study support the view that a simple ban on sub-therapeutic levels of feed antibiotics as growth promoters will not fundamentally eliminate the antibiotic resistance concerns (Looft et al., 2012). Therefore, a new effective approach to prevent dysbiosis and to detoxify LPS endotoxin needs to be developed in order to replace the sub-therapeutic levels of feed antibiotic and pharmacological levels of Cu and Zn uses in swine production and minimize the antimicrobial resistance threat to public health.

Development of alternative strategies in terms of nutritive and non-nutritive feed supplements and or additives to replace the sub-therapeutic levels of feed antibiotic in monogastric food animal production has been a hot topic of research for the past decade and this is reflected by a series of reviews of literature reports (Stein, 2002; Pettigrew, 2006; Lallès et al., 2007; Heo et al., 2013; Fan, 2013; Pluske, 2013). Current alternative strategies can be categorized into two groups, including the strategies that improve host animal growth and gut mucosal immunity and the strategies that directly or indirectly control pathogenic bacterial proliferation in the gut environment. Examples of the first category strategies include the use of rapidly digestible sugar supplements such as crystalline lactose and dry whey powder for optimal growth performances in weanling pigs (Mahan and Newton, 1993; Stein, 2002; Hayhoe, 2012). Several strategies have been developed to directly improve gut mucosal growth and functions, including using sweeteners (Shirazi-Beechey et al., 2011), gut trophic crystalline amino acids and short peptides (e.g., L-glutamine) (Wu et al., 1996; Wang et al., 2008; Fan, 2013), growth factors such as recombinant lactoferrin and epidermal growth factor (Fan, 2013), as well as positive immunomodulatory compounds such as β-glucans (Hayhoe, 2012) and omega-3 polyunsaturated fatty acids (Fan, 2013). A great deal of research efforts have been devoted to develop various prebiotic, i.e., non-viscous soluble fibers, and probiotic supplements to promote healthy gut microbiota and improve host weanling pig health and growth (Pettigrew, 2006; Fan, 2014). Examples of the second category strategies include the formulation of low-crude protein diets by using crystalline free amino acids to reduce pathogenic bacterial proliferation (Le et al., 2005; Bauer et al., 2006; Gloaguen et al., 2014), dietary supplementation of organic acids (Kirchgessner and Roth, 1998; Partanen and Mroz, 1999; Fan, 2013) and dietary inclusion of pathogen-specific egg-white antibody proteins and spray-dried plasma proteins (Marquardt et al., 1999; Stein, 2002; Pettigrew, 2006; Fan, 2013). Although pharmacological levels of Cu and Zn are shown to be effective to promote pig growth, their dietary supplementations pose detrimental environmental concerns (Fan, 2013; Heo et al., 2013). More recent work has provided evidence to show that pharmacological levels of Cu and Zn used concomitantly induce certain types of antibiotic resistance (Cavaco et al., 2011).

The general key limitations associated with above reviewed alternative common strategies are that these strategies do not always and consistently prove to be effective under all tested conditions and nor do these strategies effectively disinfect pathogenic bacteria and or detoxify LPS endotoxin. It is essential to develop an effective strategy for detoxification of LPS endotoxin and resolve the gut health challenge facing the monogastric food animal production.

4. Metabolic endotoxemia and efficiency of nutrient utilization

Metabolic endotoxemia is defined as a two-to threefold increase in circulating endotoxin concentrations above normal detectable levels (Kaliannan et al., 2013). Normal blood circulating levels of endotoxin concentrations, when expressed as an endotoxin unit (EU), are 0.1 to 0.2 EU/mL plasma reported in rodents (Ghoshal et al., 2009; Kaliannan et al., 2013) and humans (Pendyala et al., 2012). Metabolic endotoxemia leads to low-grade systemic inflammation, as further manifested by increased serum levels of pro-inflammatory cytokines such as interleukin (IL)-1, IL-6 and tumor necrosis factor-alpha (TNF-α) and enhanced gut permeability, causing accelerated translocation of endotoxin and resulting in a vicious cycle of endotoxemia (Kaliannan et al., 2013). In human nutrition and health, high-fat Western diets induced metabolic endotoxemia and contributed to the development of metabolic syndrome (Ghoshal et al., 2009; Pendyala et al., 2012; Kaliannan et al., 2013). Paradoxically, metabolic endotoxemia is poorly defined in monogastric food animals for their nutrition and metabolic research especially under practical swine production conditions. Measurements of plasma concentrations of endotoxin are no longer a technical limitation and assay kits for this type of measurements are readily available from commercial sources.

Poor energy efficiency and low profit margin of swine production is known to be associated with very low efficiency of whole body total nitrogen, i.e., crude protein (CP), retention during the post-weaning pig growth and detrimental impacts on the environment in swine production (Fan et al., 2006). Whole body efficiency of nitrogen utilization in swine declines dramatically from the suckling (83%) to the finishing phase (51%), and this is largely because gastrointestinal endogenous nitrogen and catabolic nitrogen losses are dramatically augmented due to genetic programming and changes in diets and sanitary conditions (Fan et al., 2006). Efficiency of whole body nitrogen utilization is low during most of the post-weaning production growth in swine (Fan, 2013). Systemic inflammation has been well documented to affect efficiency of nutrient utilization in monogastric food animals (Klasing, 1988). Gut luminal endotoxin and inflammation is inevitably, in part, responsible for the increased gastrointestinal endogenous nitrogen loss (Fan et al., 2006), since endotoxemia leads to increases in visceral organ protein synthetic activities (Orellana et al., 2004). Systemic endotoxin is also demonstrated to dramatically increase muscle protein catabolism (Orellana et al., 2012). Majority of current literature reports regarding effects of endotoxin on swine nutrition and metabolism have been conducted with acute (Webel et al., 1997; De Ridder et al., 2012) or chronic intraperitoneal injection (i.p.) of LPS models (Orellana et al., 2002; Kim et al., 2012; Rakhshandeh and de Lange, 2012). Nevertheless, it can be concluded that the presence of endotoxin partially accounts for the poor efficiency of nutrient utilization, low profit margin and some of the major environmental sustainable issues facing monogastric food animal of poultry and swine production. Thus, it is essential to develop effective and direct strategies for mitigation of the detrimental impact of enteric pathogenic bacterial toxins such as LPS endotoxin that are challenging the gut health in intensive monogastric food animal production.

5. Novel and disruptive biological strategies to improve monogastric food animal gut health

Novel and disruptive strategies have been effectively developed and used in intensive monogastric food animal nutrition and production including the dietary supplementations of purified trace mineral and vitamin supplements (NRC, 1998, 2012), the use crystalline limiting essential and non-essential amino acids in formulating low-protein diets (Gloaguen et al., 2014), and the application of phytase technologies for resolving the concern of poor efficiency of phosphorus utilization (Simons et al., 1990; Golovan et al., 2001; Cowieson et al., 2006). It is apparent from the above discussions that novel strategies need to be developed to directly and effectively disrupt the key step or component from

pathogenic bacteria that causes immunological and infectious responses to the host. It is rather challenging to completely disinfect and sterilize pathogenic bacteria without affecting commensal microflora in the gut environment such as the current practices of dietary supplementations of feed antibiotics, organic acids, and pharmacological levels of copper and zinc. Three potentially disruptive biological strategies may be further developed to specifically target at the major pathogenic bacterial endotoxin LPS, including regulation of the bacterial membrane polysaccharide biosynthesis pathway, lipolysis of the LPS lipid A moiety by unique lipases & esterases, and dephosphorylation of the LPS diphosphoryl lipid A moiety by alkaline phosphatases. These three potential strategies are further elaborated below.

Both Gram-negative and Gram-positive bacterial cell surfaces anchor a large number of polysaccharides and these polysaccharides play important roles in a wide variety of biological processes such as adhesion to biotic and abiotic substrata, motility, biofilm formation, cell–cell communication and immune system interaction and activation (Islam and Lam, 2014). For example, polysaccharides represent a large core of the Gram-negative bacterial endotoxin LPS. Thus, understanding of regulation of the bacterial membrane polysaccharide biosynthesis pathway has been a major focus of molecular microbiology research to develop pharmacological strategies for combating bacterial immune activation and infections.

Pathogenic bacterial endotoxin LPS typically consist of two distinct structural features, including a hetropolysaccharide chain and a lipid A moiety. The lipid A moiety has been established as the main toxic determinant of LPS in mediating immune responses via TLR4 (Ahn et al., 2004). It has been well established that the presence of the three structural components in the lipid A moiety is essential for LPS to induce their toxic effects and these include both phosphate groups, the glucosamine disaccharide, and all the fatty acyl chains especially the 2'-lauroyl and 3'-myristoyl acyloxy residues (Ahn et al., 2004). Detoxification of bacterial LPS via deacylation and lipolysis of the lipid A moiety through leukocyte, liver and kidney acyloxyacyl hydrolase (AOAH), an endogenous lipase, was reported (Munford and Hunter, 1992; Shao et al., 2007). Ahn et al. (2004) demonstrated that some of the screened microbial lipases and esterases were considerably more efficacious than mammalian pancreatic and hepatic lipases and esterases in hydrolytically releasing laurate and myristate from LPS. These results have two important gut health implications. Firstly, the endogenous lipases and esterases in animals are likely limited in their efficacy in detoxification of bacterial LPS via deacylation and lipolysis of the lipid A moiety. Secondly, development of novel and efficacious lipases and esterases as exogenous enzyme supplements may be one of the alternative disruptive strategies for detoxification of enteric endotoxin LPS in protecting gut health of food animals.

Intestinal alkaline phosphatase (IAP) is one of the most abundant brush border membrane-bound proteins (McComb et al., 1979; Fan et al., 1999). Its classic physiological functions include intestinal absorption of lipids, regulation of duodenal secretions and pH, detoxification of bacterial endotoxins, and regulation of the transmucosal passage of bacteria (Lallès, 2010). The most important physiological function of alkaline phosphatases is their unique biochemical property of detoxification of the endotoxin LPS via dephosphorylation. McComb et al. (1979) were the first to propose the potential role of alkaline phosphatases in dephosphorylation of potential toxins. Poelstra et al. (1997a,b) were the first to demonstrate that alkaline phosphatases were responsible for detoxification of the endotoxin LPS via dephosphorylation *in vivo* studies with rats. Bentala et al. (2002) and Koyama et al. (2002) subsequently showed that the removal of phosphate from the lipid A moiety in LPS was the mechanism of endotoxin detoxification by alkaline phosphatases. Various physiological and dietary factors are shown to affect the expression and functionality of IAP in animals (Lallès, 2010). Inadequate supply of enteral nutrients resulted in abnormal expression and functionality of IAP, which was demonstrated in rodent models (Goldberg et al., 2008). Inflammation and pro-inflammatory cytokines inhibited intestinal alkaline phosphatase expression (Lallès, 2010). Intestinal alkaline phosphatases prevented metabolic endotoxemia, low-grade inflammation and metabolic syndrome in mice fed high-fat diets (Kaliannan et al., 2013). Our previous studies shown that early weaning in piglets dampened the maximal enzyme activity and the digestive capacity of the IAP by down-regulation of this gene at the transcriptional, translational and post-translational levels with considerable decreases in the apical IAP abundance and affinity in the proximal jejunum (Lackeyram et al., 2010). Therefore, development of novel and efficacious alkaline phosphatases as exogenous enzyme supplements may be another alternative disruptive strategy for detoxification of enteric endotoxin LPS in protecting gut health of food animals.

6. Conclusions

Wide use of feed antibiotics as growth promoters in monogastric food animal production in the past six decades has contributed to the development of antimicrobial resistance, threatening to the world public health. Efficacy of currently available alternative feed additives in replacing feed antibiotics to maintain productivity and gut health is inconsistent dependent upon experimental conditions. Disruptive biological strategies such as novel lipases & esterases and alkaline phosphatases as exogenous feed enzymes need to be developed in replacement of feed antibiotic growth promoters to directly and effectively detoxify the endotoxin LPS and to maintain gut health and performances in monogastric food animals.

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References

Alltech. Global feed survey. 2015. http://www.alltech.com/sites/default/files/ [online].

Ahn JM, Wentworth Jr. P, Janda KD. Probing lipase/esterase libraries for lipid A hydrolasesdiscovery of biocatalysts for the detoxification of bacterially-expressed recombinant protein. Chem Commun 2004:364–5.

Aminov RI, Mackie RI. Evolution and ecology of antibiotic resistance genes. FEMS Microbiol Lett 2007;271:147–61.

Bauer E, Williams BA, Smidt H, Mosenthin R, Verstegen MW. Influence of dietary components on development of the microbiota in single-stomached species. Nutr Res Rev 2006;19:63–78.

Bentala H, Verweij WR, Huizinga-Van der Vlag A, van Loenen-Weemaes AM, Meijer DK, Poelstra K. Removal of phosphate from lipid A as a strategy to detoxify lipopolysaccharide. Shock 2002;18:561–6.

Buchanan JB, Johnson RW. Regulation of food intake by inflammatory cytokines in the brain. Neuroendocrinology 2007;86:183–90.

Casewell M, Friis C, Marco E, McMullin P, Phillips I. The European ban on growth-promoting antibiotics and emerging consequences for human and animal health. J Antimicrob Chemother 2003;52:159–61.
Cavaco LM, Hasman H, Aarestrup FM. Zinc resistance of Staphylococcus aureus of animal origin is strongly associated with methicillin resistance. Vet Microbiol 2011;150:344–8.
Chee-Sanford JC, Mackie RI, Koike S, Krapac IG, Lin YF, Yannarell AC, et al. Fate and transport of antibiotic residues and antibiotic resistance genes following land application of manure waste. J Environ Qual 2009;38:1086–108.
Chen KT, Malo MS, Moss AK, Zeller S, Johnson P, Ebrahimi F, et al. Identification of specific targets for the gut mucosal defense factor intestinal alkaline phosphatase. Am J Physiol Gastrointest Liver Physiol 2010;299:G467–75.
Cowieson AJ, Hruby M, Pierson EE. Evolving enzyme technology: impact on commercial poultry nutrition. Nutr Res Rev 2006;19:90–103.
Cromwell GL. Antimicrobial and promicrobial agents. In: Lewis AJ, Southern LL, editors. Swine nutrition. Boca Raton, FL: CRC Press LLC; 2001. p. 401–26.
Dantzer R, O'Connor JC, Freund GG, Johnson RW, Kelley KW. From inflammation to sickness and depression: when the immune system subjugates the brain. Nat Rev Neurosci 2008;9:46–56.
Davies JE, Roberts MC, Levy SB, Miller GH, OBrien TF, Tenover TC. Antimicrobial resistance: an ecological perspective. Report from Colloquium Held in San Juan. Washington, DC: Puerto Rico. Am. Acad. Microbiol.; 1999.
De Ridder K, Levesque CL, Htoo JK, de Lange CF. Immune system stimulation reduces the efficiency of tryptophan utilization for body protein deposition in growing pigs. J Anim Sci 2012;90:3485–91.
Diamond J. Evolution, consequences and future of plant and animal domestication. Nature 2002;418:700–7.
Erridge C, Bennett-Guerrero E, Poxton IR. Structure and function of lipopolysaccharides. Microbes Infect 2002;4:837–51.
Fan MZ. Concepts of animal nutrition. 2nd ed. Boston MA: Pearson; 2014.
Fan MZ. Swine nutrition and environment. In: Chiba L, editor. Sustainable swine nutrition. John Wiley & Sons, Inc.; 2013. p. 365–411.
Fan MZ, Adeola O, Asem EK. Characterization of brush border membrane-bound alkaline phosphatase activity in different segments of the porcine small intestine. J Nutr Biochem 1999;10:299–305.
Fan MZ, Chiba LI, Matzat PD, Yang X, Yin YL, Mine Y, et al. Measuring synthesis rates of nitrogen-containing polymers by using stable isotope tracers. J Anim Sci 2006;84 Suppl:E79-E93.
Fan MZ, Kim SW, Applegate TJ, Cervantes M. Nonruminant nutrition symposium: understanding protein synthesis and degradation and their pathway regulations. J Anim Sci 2008;86 E. Suppl:E1-E2.
Fard RM, Heuzenroeder MW, Barton MD. Antimicrobial and heavy metal resistance in commensal enterococci isolated from pigs. Vet Microbiol 2011;148:276–82.
Forslund K, Sunagawa S, Kultima JR, Mende DR, Arumugam M, Typas A, et al. Country-specific antibiotic use practices impact the human gut resistome. Genome Res 2013;23:1163–9.
Gaskins HR, Collier CT, Anderson DB. Antibiotics as growth promotants: mode of action. Anim Biotechnol 2002;13:29–42.
Ghoshal S, Witta J, Zhong J, de Villiers W, Eckhardt E. Chylomicrons promote intestinal absorption of lipopolysaccharides. J Lipid Res 2009;50:90–7.
Gloaguen M, Le Floc'h N, Corrent E, Primot Y, van Milgen J. The use of free amino acids allows formulating very low crude protein diets for piglets. J Anim Sci 2014;92:637–44.
Goldberg RF, Austen WG, Zhang X, Munene G, Mostafa G, Biswas S, et al. Intestinal alkaline phosphatase is a gut mucosal defense factor maintained by enteral nutrition. Proc Natl Acad Sci U S A 2008;105:3551–6.
Golovan SP, Meidinger RG, Ajakaiye A, Cottrill M, Wiederkehr MZ, Barney DJ, et al. Pigs expressing salivary phytase produce low-phosphorus manure. Nat Biotechnol 2001;19:741–5.
Gorbach SL. Antimicrobial use in animal feed–time to stop. N Engl J Med 2001;345:1202–3.
Groenen MA, Archibald AL, Uenishi H, Tuggle CK, Takeuchi Y, Rothschild MF, et al. Analyses of pig genomes provide insight into porcine demography and evolution. Nature 2012;491:393–8.
Guenther S, Grobbel M, Lubke-Becker A, Goedecke A, Friedrich ND, Wieler LH, et al. Antimicrobial resistance profiles of Escherichia coli from common European wild bird species. Vet Microbiol 2010;144:219–25.
Hayhoe M. Efficacy of prebiotics and β-glucan in modulation of growth and alkaline phosphatase kinetics in the weanling pig. MSc thesis. Guelph, Ontario, Canada: University of Guelph; 2012.
Heo JM, Opapeju FO, Pluske JR, Kim JC, Hampson DJ, Nyachoti CM. Gastrointestinal health and function in weaned pigs: a review of feeding strategies to control post-weaning diarrhoea without using in-feed antimicrobial compounds. J Anim Physiol Anim Nutr Berl 2013;97:207–37.
Islam ST, Lam JS. Synthesis of bacterial polysaccharides via the Wzx/Wzy-dependent pathway. Can J Microbiol 2014;60:697–716.
Johnson JR, Kuskowski MA, Smith K, O'Bryan TT, Tatini S. Antimicrobial-resistant and extraintestinal pathogenic Escherichia coli in retail foods. J Infect Dis 2005;191:1040–9.
Johnson RW. Immune and endocrine regulation of food intake in sick animals. Domest Anim Endocrinol 1998;15:309–19.
Kaliannan K, Hamarneh SR, Economopoulos KP, Nasrin Alam S, Moaven O, Patel P, et al. Intestinal alkaline phosphatase prevents metabolic syndrome in mice. Proc Natl Acad Sci U S A 2013;110:7003–8.
Kelley KW, Bluthé R-M, Dantzer R, Zhou J-H, Shen W-H, Johnson RW, et al. Cytokine-induced sickness behavior. Brain Behav Immun 2003;17:112–8.
Kim JC, Mullan BP, Frey B, Payne HG, Pluske JR. Whole body protein deposition and plasma amino acid profiles in growing and/or finishing pigs fed increasing levels of sulfur amino acids with and without Escherichia coli lipopolysaccharide challenge. J Anim Sci 2012;90(Suppl. 4):362–5.
Kirchgessner M, Roth RZ. Organic acids as feed additives for young pigs: nutritional and gastrointestinal effects. J Anim Feed Sci 1998;7:25–33.
Klasing KC. Nutritional aspects of leukocytic cytokines. J Nutr 1988;118:1436–46.
Koike S, Krapac IG, Oliver HD, Yannarell AC, Chee-Sanford JC, Aminov RI, et al. Monitoring and source tracking of tetracycline resistance genes in lagoons and groundwater adjacent to swine production facilities over a 3-year period. Appl Environ Microbiol 2007;73:4813–23.
Koyama I, Matsunaga T, Harada T, Hokari S, Komoda T. Alkaline phosphatases reduce toxicity of lipopolysaccharides in vivo and in vitro through dephosphorylation. Clin Biochem 2002;35:455–61.
Kulp A, Kuehn MJ. Biological functions and biogenesis of secreted bacterial outer membrane vesicles. Annu Rev Microbiol 2010;64:163–84.
Lackeyram D, Yang C, Archbold T, Swanson KC, Fan MZ. Early weaning reduces small intestinal alkaline phosphatase expression in pigs. J Nutr 2010;140:461–8.
Lallès JP. Intestinal alkaline phosphatase: multiple biological roles in maintenance of intestinal homeostasis and modulation by diet. Nutr Rev 2010;68:323–32.
Lallès JP, Bosi P, Smidt H, Stokes CR. Nutritional management of gut health in pigs around weaning. Proc Nutr Soc 2007;66:260–8.
Le PD, Aarnink AJ, Ogink NW, Becker PM, Verstegen MW. Odour from animal production facilities: its relationship to diet. Nutr Res Rev 2005;18:3–30.
Levy SB. Multidrug resistance – a sign of the times. N Engl J Med 1998;338:1376–8.
Looft T, Johnson TA, Allen HK, Bayles DO, Alt DP, Stedtfeld RD, et al. In-feed antibiotic effects on the swine intestinal microbiome. Proc Natl Acad Sci U S A 2012;109:1691–6.
Mahan DC, Newton EA. Evaluation of feed grains with dried skim milk and added carbohydrate sources on weanling pig performance. J Anim Sci 1993;71:3376–82.
Manges AR, Johnson JR. Food-borne origins of Escherichia coli causing extraintestinal infections. Clin Infect Dis 2012;55:712–9.
Marquardt RR, Jin LZ, Kim JW, Fang L, Frohlich AA, Baidoo SK. Passive protective effect of egg-yolk antibodies against enterotoxigenic Escherichia coli K88+ infection in neonatal and early-weaned piglets. FEMS Immunol Med Microbiol 1999;23:283–8.
McComb R, Bowers J, Posen S, editors. Alkaline phosphatase. New York: Plenum Press; 1979.
McCracken VJ, Simpson JM, Mackie RI, Gaskins HR. Molecular ecological analysis of dietary and antibiotic-induced alterations of the mouse intestinal microbiota. J Nutr 2001;131:1862–70.
Mulder IE, Schmidt B, Lewis M, Delday M, Stokes CR, Bailey M, et al. Restricting microbial exposure in early life negates the immune benefits associated with gut colonization in environments of high microbial diversity. PLoS One 2011;6:e28279.
Mulder IE, Schmidt B, Stokes CR, Lewis M, Bailey M, Aminov RI, et al. Environmentally-acquired bacteria influence microbial diversity and natural innate immune responses at gut surfaces. BMC Biol 2009;7:79.
Munford RS, Hunter JP. Acyloxyacyl hydrolase, a leukocyte enzyme that deacylates bacterial lipopolysaccharides, has phospholipase, lysophospholipase, diacylglycerollipase, and acyltransferase activities in vitro. J Biol Chem 1992;267:10116–21.
National Research Council. Nutrient requirements of Swine10. Washington, DC: National Academies Press; 1998.
National Research Council. Nutrient requirements of swine11th. Washington, D.C.: National Academies Press; 2012.
Orellana RA, O'Connor PM, Nguyen HV, Bush JA, Suryawan A, Thivierge MC, et al. Endotoxemia reduces skeletal muscle protein synthesis in neonates. Am J Physiol Endocrinol Metab 2002;283:E909–16.
Orellana RA, Kimball SR, Nguyen HV, Bush JA, Suryawan A, Thivierge MC, et al. Regulation of muscle protein synthesis in neonatal pigs during prolonged endotoxemia. Pediatr Res 2004;55:442–9.
Orellana RA, Suryawan A, Wilson FA, Gazzaneo MC, Fiorotto ML, Nguyen HV, et al. Development aggravates the severity of skeletal muscle catabolism induced by endotoxemia in neonatal pigs. Am J Physiol Regul Integr Comp Physiol 2012;302:R682–90.
Partanen KH, Mroz Z. Organic acids for performance enhancement in pig diets. Nutr Res Rev 1999;12:117–45.
Pendyala S, Walker JM, Holt PR. A high-fat diet is associated with endotoxemia that originates from the gut. Gastroenterology 2012;142:1100–1 e2.
Pettigrew JE. Reduced use of antibiotic growth promoters in diets fed to weanling pigs: dietary tools, part 1. Anim Biotechnol 2006;17:207–15.
Pié S, Lallès JP, Blazy F, Laffitte J, Sève B, Oswald IP. Weaning is associated with an upregulation of expression of inflammatory cytokines in the intestine of piglets. J Nutr 2004;134:641–7.
Pluske JR. Feed- and feed additives-related aspects of gut health and development in weanling pigs. J Anim Sci Biotechnol 2013;4:1.
Poelstra K, Bakker WW, Klok PA, Hardonk MJ, Meijer DK. A physiologic function for alkaline phosphatase: endotoxin detoxification. Lab Invest 1997a;76:319–27.
Poelstra K, Bakker WW, Klok PA, Kamps JA, Hardonk MJ, Meijer DK. Dephosphorylation of endotoxin by alkaline phosphatase in vivo. Am J Pathol 1997b;151:1163–9.
Poltorak A, He X, Smirnova I, Liu MY, Van Huffel C, Du X, et al. Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. Science 1998;282:2085–8.

Pond WG, Lei XG. Of pigs and people. In: Lewis AJ, Southern LL, editors. Swine nutrition. Boca Raton, Florida: CRC Press LLC; 2001. p. 3–18.
Rakhshandeh A, de Lange CF. Evaluation of chronic immune system stimulation models in growing pigs. Animal 2012;6:305–10.
Rietschel ET, Kirikae T, Schade FU, Mamat U, Schmidt G, Loppnow H, et al. Bacterial endotoxin: molecular relationships of structure to activity and function. FASEB J 1994;8:217–25.
Salzman AL, Wang H, Wollert PS, Vandermeer TJ, Compton CC, Denenberg AG, et al. Endotoxin-induced ileal mucosal hyperpermeability in pigs: role of tissue acidosis. Am J Physiol 1994;266:G633–46.
Schmidt B, Mulder IE, Musk CC, Aminov RI, Lewis M, Stokes CR, et al. Establishment of normal gut microbiota is compromised under excessive hygiene conditions. PLoS One 2011;6:e28284.
Shao B, Lu M, Katz SC, Varley AW, Hardwick J, Rogers TE, et al. A host lipase detoxifies bacterial lipopolysaccharides in the liver and spleen. J Biol Chem 2007;282:13726–35.
Shirazi-Beechey SP, Moran AW, Bravo D, Al-Rammahi M. Nonruminant nutrition Symposium: intestinal glucose sensing and regulation of glucose absorption: implications for swine nutrition. J Anim Sci 2011;89:1854–62.
Simons PC, Versteegh HA, Jongbloed AW, Kemme PA, Slump P, Bos KD, et al. Improvement of phosphorus availability by microbial phytase in broilers and pigs. Br J Nutr 1990;64:525–40.
Stein HH. Experience of feeding pigs without antibiotics: a European perspective. Anim Biotechnol 2002;13:85–95.
Umber JK, Bender JB. Pets and antimicrobial resistance. Vet Clin North Am Small Anim Pract 2009;39:279–92.
Varley, Wiseman. The weaner pig: nutrient and management. New York, NY: CABI International; 2001.
Visek WJ. The mode of growth promotion by antibiotics. J Anim Sci 1978;46:1447–69.
Wang J, Chen L, Li P, Li X, Zhou H, Wang F, et al. Gene expression is altered in piglet small intestine by weaning and dietary glutamine supplementation. J Nutr 2008;138:1025–32.
Webel DM, Finck BN, Baker DH, Johnson RW. Time course of increased plasma cytokines, cortisol, and urea nitrogen in pigs following intraperitoneal injection of lipopolysaccharide. J Anim Sci 1997;75:1514–20.
World Health Organization WHO. Antimicrobial resistance: Global report on surveillance. 2014.
Wright GD. The antibiotic resistome: the nexus of chemical and genetic diversity. Nat Rev Microbiol 2007;5:175–86.
Wu G, Meier SA, Knabe DA. Dietary glutamine supplementation prevents jejunal atrophy in weaned pigs. J Nutr 1996;126:2578–84.