SYMPOSIUM: Impact of the systemic response to stressors and subclinical and clinical infection on intestinal barrier function and growth in pigs

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Abstract

Chronic exposure to clinical/subclinical infection and stressors compromises intestinal barrier function and activates a systemic response which reduces the rate of protein deposition and growth efficiency. The systemic response influences performance of pigs in two ways: alteration of nutrient partitioning and eicosanoid mediator-induced neurological responses to infection such as anorexia. The roles of nutrition to tackle both routes of the systemic response are reviewed. Evidence is provided that the systemic responses alter nutrient partitioning and increase requirements for tryptophan and sulphur amino acids. Nutrients that reduce either cyclooxygenase and 5-lipoxygenase activity or cyclooxygenase and lipoxygenase gene expressions, and hence minimise production of eicosanoid mediators such as prostaglandin E\(_2\) and leukotriene B\(_4\) includes omega-3 fatty acids, boron, probiotics and antioxidants. The purpose of this review is to provide an overview of several nutritional strategies to minimise impacts of chronic subclinical infection and stressors on the efficiency of pork production.

Introduction

Physiological and immunological responses to subclinical/clinical infection and nutritional and psychological stressors significantly alter intestinal barrier functions (Kim et al., 2012a) and the way pigs partition nutrients (Kim et al., 2012b). Pigs in a commercial production system are continuously exposed to various pathogens and viruses that activate the immune system at subclinical or clinical levels. Growth efficiency of pigs is significantly compromised at even subclinical level activation of the immune system due to altered nutrient partitioning, compromised intestinal barrier functions and malnutrition caused by anorexia. The systemic response of pigs to immune system activation orchestrated by mast cells drains nutrients for production of immune molecules, such as cytokines, acute phase proteins and immunoglobulins, away from deposition as lean and lipid. Furthermore, stimulation of the eicosanoid pathways by immune system activation subsequently increases production of arachidonic acid derivatives such as prostaglandin E\(_2\) (PGE\(_2\)) and leukotriene B\(_4\) (LTB\(_4\)), which are known to provoke anorexia and fever through stimulation of the neuroendocrine system (Rivest, 2010) and control the severity and duration of the immune response, respectively (Devchand et al., 1996).

Understanding the systemic responses to stressors and subclinical infection on intestinal barrier function and growth, and subsequent nutritional strategies to suppress their impact on nutrition and health of pigs, is one of the least explored areas in pig nutrition yet they can have significant influences on health, welfare and growth efficiency of pigs. Therefore, this review will focus on the systemic response to stressors and subclinical infection on intestinal barrier function and growth, and will present possible nutritional strategies that can suppress or minimise the impact of the systemic response to stressors and subclinical infection.

Acute and systemic immune responses to subclinical and clinical intestinal infection

Development of mucosal immunity and immunophysiology of the gut-associated lymphoid tissue (GALT) was recently reviewed by Emery and Collins (2011). Briefly, pathogens and antigens are entering the system either through microfold (M) cells in the Peyer’s patch or transcellular and paracellular pathways in the intestinal epithelium. The antigens and pathogens that enter through M cells are then transferred to dendritic cells (DC) where toll-like receptors (TLRs) are expressed. Induction of inflammation then stimulates maturation of DC and antigen/pathogen information is presented to T cells in the Peyer’s patch. Alternatively, antigens or antigen loaded DC enter into mesenteric lymph nodes where subsequent T-cell recognition occurs. Antigens translocated through epithelial paracellular or transcellular pathways are disseminated either to the mesenteric lymph nodes via antigen presenting cells or the peripheral lymph nodes and interact with T cells (Mowat, 2003).

In the acute phase of pathogen invasion, locally existing sentinel cells like macrophages and mast cells are immediately activated and release pro-inflammatory cytokines such as interleukin (IL)-1, IL-6 and...
tumor necropsy factor (TNF)-α and increase neutrophils, monocytes and lymphocytes to the site of inflammation (Goddeeris et al., 2002). One of the most important acute phase responses after inflammation is increased recruitment of phagocytes and subsequent specific immune response against the pathogens and antigens (Goddeeris et al., 2002). Phagocytes such as neutrophils, macrophages and mast cells release proteases and reactive oxygen species in the process of phagocytosis of antigens which causes irreversible tissue damage to the host (Wyman and Schneiter, 2008). Unlike lymphocytes where T-cell and B-cell receptors recognise antigens, neutrophil-driven phagocytosis and stimulation of mast cells are initiated by immunoglobulin (Ig) receptors (Wyman and Schneiter, 2008). Immunoglobulin receptors (IgG receptors in neutrophils and macrophages and IgE receptors in mast cells) then stimulate intracellular protein kinases which increase intracellular Ca^{2+} concentration. Increased intracellular Ca^{2+} concentration stimulates degranulation and production of eicosanoid mediators via activation of phospholipase 2 (Irvine, 2003; Wyman and Schneiter, 2008).

Among the many types of leukocytes, mast cells have a central role in acute- and systemic-immune response and have been studied extensively (Abraham and St John, 2010). Mast cells can directly recognise pathogens through recognition of common patterns of pathogens through pattern recognition receptors such as TLRs or binding of specific-pathogen associated antibodies (Supajatura et al., 2001; Abraham and St John, 2010). Upon recognition, mast cells have the ability to respond to the invasion in three different mechanisms. First, mast cells contain heterogeneous tryptases and chymases, which are degranulated when pathogens/antigens are recognised. For example, in vitro studies showed that co-incubation with commensal bacteria such as non-pathogenic E. coli, Bifidobacteria and Lactobacillus did not induce mast cell degranulation, while co-incubation with pathogenic E. coli induced mast cell degranulation through interaction between the E. coli adhesion protein and CD48 on mast cells (reviewed in Wesolowski and Paumet, 2011). Mast cells have the ability to modify their phenotype during the course of infection and can replenish their granules after degranulation to memorise the antigens/pathogens to control re-infection (Abraham and St John, 2010). Second, mast cells have the ability to serve as a phagocyte along with macrophages, dendritic cells and neutrophils. Mast cells internalise many types of pathogens through IgG receptors via direct interaction with bacterial adhesion, and the cytosolic lysosomes use reactive oxygen species and acidification process for phagocytosis (Wesolowski and Paumet, 2011). Thirdly, upon pathogen recognition, mast cells secret many cellular mediators including proinflammatory cytokines and regulate innate and acquired immune functions. Mast cell-derived proinflammatory cytokines such as histamine, IL-6 and TNF-α are secreted shortly after bacterial infection and are known to recruit neutrophils and DC to the infection sites and lymph nodes during E. coli infection (Weisloowski and Paumet, 2012). Mast-cell derived IL-4 and eicosanoids such as LTβ4 enhance recruitment of macrophages for intracellular phagocytosis and increase vascular permeability of the endothelial cells and oedema at the site of infection. Mast cells are also able to communicate with epithelial cells, T-cells and B-cells and increase mucus production, T cell chemotaxis and lymphocyte retention in draining lymph nodes to improve antibody production, respectively (Abraham and St John, 2010). Initiation of adaptive immunity is caused by mast-cell derived TNF-α which increases monocytes-derived antigen presenting DC in the draining lymph nodes and stimulate antibody production through T- and B-cells. Mast cells are then sensitised with the produced pathogen-specific antibodies (pathogen specific IgG or IgE) through Fc receptors, which are used for adaptive immune function (Abraham and St John, 2010).

One of the most important effects of mast cell stimulation is increased eicosanoid production, such as PGE_{2} and LTB_{4}, that induce infection-associated anorexia (Wyman and Schneiter, 2008) and control the duration of inflammation, respectively (Devchand et al., 1996). Production of eicosanoid mediators in the cell and nuclear membranes is initiated through the action of phospholipase A_{2} or C which respectively convert membrane-bound phospholipids and diacylglycerols to arachidonic acids. Arachidonic acids are then converted to either PGE_{2} or LTB_{4} by cyclooxygenase and lipoxygenase, respectively (Folco and Murphy, 2006; Wyman and Schneiter, 2008; Kalinski 2012). In human medicine, steroids and non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin are used to directly inhibit phospholipase and cyclooxygenase activities (Wyman and Schneiter, 2008). Another route that can modulate the impact of inflammation is by facilitating LTB_{4} degradation since its clearance in the system is directly associated with the severity and duration of the inflammatory response (Devchand et al., 1996). In this context Devchand et al. (1996) used a mouse ear swelling test where LTB_{4} was topically applied to the ear, and then measured ear thickness as an index of inflammatory response. Unlike normal mice, peroxisome proliferator-activated receptors-α (PPARα)–knockout mice significantly increased the severity and duration of the inflammatory response. The PPARα is a nuclear transcription factor that facilitates systemic catabolism of LTB_{4} (Narala et al., 2010). Therefore, the PPARα knockout mice which lack the ability to catabolise LTB_{4} extended the severity and duration of inflammation. Catabolism of LTB_{4} is facilitated by PPARα that activates microsomal ω- and peroxisomal β-oxidation pathways and primarily

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occurs in the immune cells in the inflammation site and also in the hepatocytes (Devchand et al., 1996; Yokomizo et al., 2001).

**Acute and systemic immune responses to stressors**

Pigs in commercial production systems encounter numerous socio-physiological and environmental stressors that trigger acute and systemic immune responses. Stress stimulates the hypothalamus-pituitary-adrenal axis and releases a 41-amino acid peptide, corticotrophin-releasing factor (CRF), from the paraventricular nucleus of the hypothalamus (Moese et al., 2007; Salak-Johnson and McGlone, 2007; Teitelbaum et al., 2008). The CRF then binds to two G-protein-coupled receptors, CRF-R1 and CRF-R2, in the peripheral tissues and stimulates acute and systemic immune responses (Moese et al., 2007; Teitelbaum et al., 2008). For example, the stress of weaning (Moese et al., 2007), especially if done before 21 d of age (Smith et al., 2010), significantly increased jejunal and colonic expression of CRF and CRF receptors. Increased release of CRF also stimulates mast cells and Smith et al. (2010) demonstrated that pigs weaned at 18 d increased their CRF contents, mast cell numbers and mast cell tryptases' expression in the jejunum compared with pigs weaned at 23 d. Moreover, in a Ussing Chamber model, it was demonstrated that exposure to CRF increases TNF-α and tryptases' activity and the proportion of the degranulated mast cell in the ileum of 6-8-week old pigs (Overman et al., 2012). In a pig study, a four-hour transportation significantly increased natural killer (NK) cell cytotoxicity in pigs with intermediate and submissive social status compared with socially dominant pigs, and a significant positive correlation between NK cell cytotoxicity and plasma cortisol level was reported (McGlone et al., 1993). This particular study indicates that susceptibility to social stressors is mainly dependent on the social status of pigs, and pigs in low hierarchy are prone to the CRF-induced immune response.

**Impact of acute and systemic immune response on intestinal barrier function**

Subclinical or clinical inflammation and socio-physical and environmental stressors such as weaning, transportation, mixing, isolation, heat and cold stress stimulates mast cells either directly or through release of CRF and negatively affects epithelial barrier function (Berkes et al., 2003; Overmen et al., 2012).

Enteric pathogens and antigens in the intestinal lumen enter through transcellular or paracellular pathways, and the presence of enteric pathogens and antigens in the intestinal lumen significantly influence intestinal permeability. Although mechanisms behind the inflammation-induced decrease in transcellular permeability is not well understood, mechanisms for inflammation- and stress-induced reductions in paracellular permeability are well studied and are primarily regulated by the tight junction between enterocytes. The major tight junction proteins are transmembrane protein complexes occludins and claudins, and cytosolic proteins zonula occludins (ZO). At the apical-lateral membrane junction of the enterocytes, claudin-1 and occludin binds the two lateral membranes of the adjacent enterocytes and ZO-1, ZO-2 and ZO-3 connect the claudin-1 and occludin to cytoskeletal actins (Groschwitz and Hogan, 2009). It is known that enteric pathogens and their toxins increase paracellular permeability by altering the tight junction structure via actomyosine ring alteration or tight junction protein redistribution (Berkes et al., 2003). Clostridium difficile, enterotoxigenic Escherichia coli (ETEC) and Bacteroides fragilis alter actomyosin rings and increase paracellular permeability through depolymerising actin, myosin light chain phosphorylation and proteolysis of tight junction proteins, respectively. On the other hand Clostridium difficile, ETEC, Vibrio cholera and Clostridium perfringens redistribute tight junction proteins through mobilisation of occludin and ZO-1 away from the tight junction, occludin degradation by haemagglutinin protease, and redistribution of claudin (Berkes et al., 2003). Stress-induced release of CRF is also known to increase transcellular permeability (Teitelbaum et al., 2008) and paracellular permeability by redistribution of occludin (Overman et al., 2012). Especially in a weaner pig model, it was demonstrated that CRF increases paracellular permeability by stimulating release of TNF-α and protease from mast cells (Overman et al., 2012).

Intestinal barrier function is technically quantified by measuring the resistance to transcellular permeability (Transepithelial electrical resistance, TER; short circuit ion transport Isc) and the permeability to a macromolecule such as horseradish peroxidase, 4-KDa FITC-Dextran, 180 kDa [3H]mannitol, 5000 kDa [14C]julin or lactulose, that can only be translocated through paracellular pathway. In a mouse study, Roxas et al. (2010) demonstrated that ETEC infection decreased TER by 60% and increased 4-KDa FITC-Dextran flux four-fold in the colonic tissues at 8 d after infection. Increased CRF release observed in early weaned pigs (15 d versus 28 d) decreased TER and increased mucosal to serosal [3H]mannitol flux by 20% in the jejunal epithelium when measured at nine weeks of age (Smith et al., 2010). Pearce et al. (2012) demonstrated in a grower pig study (46 kg live weight) that acute exposure to heat stress (24 h at 35 °C, 24-43% humidity) increased paracellular permeability by two-fold and six-fold in the ileum and colon, respectively, compared with pigs in the thermo-neutral condition (21 °C, 35-
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50% humidity). The study also showed that transcellular permeability was increased by 2-fold and 30% in the ileum and colon, respectively, in the pigs acutely exposed to the heat stress. Therefore, it is clearly demonstrated that either infection or stress-induced release of CRF significantly compromises intestinal barrier function in pigs.

![Diagram of immune response](image)

**Figure 1. Acute and systemic responses to infection (from Beisel, 1991).**

**Impact of acute and systemic immune response on growth and protein deposition**

Pigs kept under commercial conditions are continuously exposed to microorganisms, and typically respond to these immune system challenges by elevated release of acute-phase proteins, cytokines, increased metabolic use of protein (i.e., synthesis of immune molecules), reduced feed intake and hence decreased protein deposition (Kim et al., 2012b). Cytokines involved in the acute and systemic response to infection and their effects on nutrient partitioning and mobilisation are illustrated in Figure 1 (Beisel, 1991). Release of pro-inflammatory cytokines from monocytes, macrophages and mast cells stimulate the hepatic uptake of amino acids and increase production of acute phase proteins (APP) in the liver. Moreover, physical, psychological and environmental stresses such as weaning, isolation, transportation and a disorderly feeding pattern are known to increase APP production through stimulation of the sympathoadrenal and hypothalamic-pituitary-adrenal axis (Pineiro et al., 2007; Soler et al., 2013). Muscle proteins are catabolised and lipolysis is increased in severe and chronic cases of inflammation or stress where circulating and hepatic amino acid and energy reserves are not meeting the increased requirements from the hepatocytes and immune cells (Johnson, 1997; Rakhshandeh and de Lange, 2011, Kim et al., 2012b). Increased muscle protein catabolism and lipolysis then eventually reduces protein and fat deposition.
(Rakhshandeh and de Lange, 2011). Apart from the increased metabolic requirements, stimulation of the central nervous system is known to reduce feed intake which exacerbates malnutrition of the immune system-activated pigs (Rakhshandeh and de Lange, 2011; Pastorelli et al., 2012a).

The consequences of subclinical levels of immune response on performance of pigs was examined recently, where weaner pigs were housed either in a disinfected room or in an uncleaned room that contained pathogens from the previous batch of pigs. In a 42-d feeding experiment, average daily gain of the pigs exposed to an unclean environment was reduced by 11-12% due to reduced feed intake (5%) and feed efficiency (7%, Pastorelli et al., 2012b; 2012c). This finding suggests that even a mild subclinical activation of the immune response has significant impacts on growth of pigs, and that both altered nutrient partitioning (feed efficiency) and the eicosanoid mediators-induced loss of appetite have contributed to the reduced daily gain. Another weaner pig study experimentally created a spectrum of ileitis by inoculating varying dose of a mucosal homogenate containing Lawsonia intracellularis (LI) (10^8 - 10^4 LI per pig; Paradis et al., 2012). Pigs inoculated with 10^8 and 10^7 LI showed clinical signs of ileitis while pigs inoculated with 10^5 and 10^4 LI did not show clinical signs of ileitis. The study found a dose-response reduction in daily gain, feed intake and feed efficiency as dose rate of LI was increased. Interestingly, this study showed that even subclinical infection with LI (pigs inoculated with 10^5 and 10^4 LI) decreased daily gain by 37%, feed intake by 21% and feed efficiency by 21%, and pigs were 3.5 kg lighter than control pigs at 21 d after inoculation.

Consequences of clinical levels of the immune response on performance of pigs have been analysed (meta-analysis) in 122 publications, which examined the effects of pathogen and disease/sanitary environment challenges on feed intake and growth response (Pastorelli et al., 2012a). Digestive bacterial infections, poor housing conditions, mycotoxicoses, parasitic infections and respiratory disease challenges reduced average growth rate by 40%, 16%, 30%, 8% and 25%, respectively. Interestingly, it was concluded that the major mechanism responsible for the reduction in growth rate was different dependent on the type of disease challenge. For example, in the case of digestive bacterial infection, parasite infection and poor housing conditions, the changes in maintenance requirement explained approximately 70-75% of growth reduction while feed intake explained 25-30% of the growth reduction. In contrast, feed intake explained 70% of the growth reduction in pigs challenged with mycotoxicosis and respiratory disease, while the changes in maintenance requirement explained 30% of the growth reduction (Pastorelli et al., 2012a). Therefore, it seems that a disease that affects intestinal structure/function such as pathogens/toxins/parasites-driven intestinal damage reduces digestion/absorption of nutrients, and hence a greater proportion of body weight loss might be caused by the compromised digestion/absorption capacity and the metabolic needs for tissue repairs, while a lower proportion of body weight loss was caused by compromised feed intake (Klasing et al., 1991; Sandberg et al., 2007; Pastorelli et al., 2012a). In addition, increased endogenous protein loss through intestinal secretion such as mucus, loss of water in case of diarrhoea and increased nutrient requirements for immune response (Sandberg et al., 2007) also increases metabolic costs and represent reduced growth independently to reduced feed intake (Pastorelli et al., 2012a). The linear-plateau and curvilinear-plateau equations developed in the meta-analysis indicate that unlike respiratory disease where pigs' feed intake and growth rate fully recovered back to the rates observed in the control pigs, growth rate of the pigs challenged with a digestive tract bacterial infection was not recovered (10% lower) back to the growth rate in the control pigs at 40 d after the infection, despite feed intake being fully recovered at around 35 d (Pastorelli et al., 2012a). This finding suggests that after 40 d of digestive bacterial infection, feed intake-independent growth reduction was observed and represents a lasting metabolic cost for maintenance of the immune system.

Chronic immune system activation significantly compromises body protein deposition as demonstrated in early studies by Williams et al. (1997a, b, c). Pigs acquired from a herd possessing antibody titers for pathogens reduced their body protein deposition by 26-28% (Williams et al., 1997a, c) and whole body nitrogen retention rate by 20% (Williams et al., 1997b) compared with pigs possessing low antibody titers. In rat studies, muscle protein synthesis was reduced by 22% while protein syntheses in the liver and spleen were increased by 2-fold and 3-fold, respectively, in rats infected with an E. coli compared with healthy rats (Breuille et al., 1994; 1998). In an experimentally-induced chronic immune system activation model, where finisher pigs (52-91 kg) were repeatedly injected with E. coli lipopolysaccharides (LPS), the rate of whole body protein deposition was 12% lower and the plasma urea content was 12% higher in immune system-activated pigs at the currently recommended levels of sulphur amino acids (SAA) than healthy pigs injected with physiological saline (see Figure 2; Kim et al., 2012b).
Aspects of nutrition to reduce impacts of a systemic immune response

Subclinical and clinical levels of immune system activation decrease daily gain and feed intake through two major mechanisms: altered nutrient partitioning and decreased appetite. Therefore, searching for the responsible nutrients that either increase the requirements at immune system activation or have the ability to minimise production of immunosuppressive eicosanoid mediators may provide useful nutritional strategies to reduce the impact of immune system activation on the growth and health status of pigs.

Amino acids

Immune system activation decreases feed efficiency by altering nutrient partitioning. Release of pro-inflammatory cytokines stimulates the liver and increases hepatic uptake of amino acids to produce immune molecules (Breuille et al., 1994; 1998). Significant amino acid catabolism in the skeletal muscle is evident in immune system-activated pigs and rats and depends on severity and duration of the inflammation (Breuille et al., 1994; 1998; Williams et al., 1997a, b). A comprehensive review on roles of individual amino acids for whole body protein deposition in immune-system-activated pigs was presented in a previous meeting (Rakhshandeh and de Lange, 2011). Briefly, despite increased metabolic demand for glutamine, arginine, phenylalanine, tyrosine and branched-chain amino acids during mild immune system activation, their requirements do not increase due to sufficient de novo synthesis or sufficient amounts present in current diets. Although only limited information is available, it is believed that threonine requirements may be increased in cases where mucus production or threonine-rich immunoglobulin production is stimulated during immune system activation since threonine is the major amino acid in mucus and some immunoglobulins (Faure et al., 2007; Rakhshandeh and de Lange, 2011).

Tryptophan (Trp) is an important amino acid that is limiting the growth of pigs during states of immune system activation because it is a precursor for kynurenine, serotonin and melatonin that significantly increase during immune system activation (Rakhshandeh and de Lange, 2011). It is reported that the Trp requirement for protein deposition is increased when the immune system is activated while the Trp requirement for maintenance was not changed (de Ridder et al., 2012). Using intramuscular injection of an E. coli lipopolysaccharide (LPS), it was demonstrated that Trp requirement increased by 7% in immune system-activated growing pigs (de Ridder et al., 2012). In weaner pigs orally infected with an enterotoxigenic strain of E. coli, increased Trp:lysine ratios from 0.18 to 0.22 (Trevisi et al., 2009a, b) and 0.26 (Capozzalo et al., 2012) improved daily gain and feed conversion efficiency, respectively.

The sulphur amino acids’ (SAA), especially cysteine, requirement is known to increase in immune system-activated pigs. Immune system activation increased cysteine synthesis in humans (Yu et al., 1993) and rats (Malmzet et al., 2000), and upregulated hepatic gene expression for cystathionine β-synthase and cystathionine γ-lyase, the enzymes involved in cysteine biosynthesis in pigs (Rakhshandeh et al., 2010b). Cysteine is mainly used for production of glutathione and taurine that prevent tissue damage from reactive oxygen and nitrogen species generated from phagocytes and leukocytes that were recruited to the infection sites (Babior, 2000; Splettstoesser and Schuff-wnerner, 2002; Wyman and Schneider, 2008; Rakhshandeh and de Lange, 2011). Furthermore, SAA are extensively used for acute phase protein synthesis and are used as a structural molecule for immune molecules such as immunoglobulins (Rakhshandeh and de Lange, 2011). Increased SAA requirement in immune system-activated pigs was well demonstrated in a growing pig study where immune system-activated pigs decreased urinary sulphur excretion while urinary nitrogen excretion was increased (Rakhshandeh et al., 2010a). This particular finding indicates that SAA are
limiting efficient utilisation of the other essential amino acids when the pigs’ immune system is activated. Recently a grower finisher pig study was conducted with an immune system activation model where two groups of pigs were repeatedly injected either with an E. coli LPS or saline to mimic the level of immune system activation experienced in commercial herds, where chronic challenges from pathogens and viruses exist. The study was designed to determine SAA requirement for maximum protein deposition in either healthy or immune-system-activated pigs (See Figure 2, Kim et al., 2012b). This study demonstrated that unlike healthy pigs, the rate of whole body protein deposition in immune system activated pigs was decreased by 12% at currently recommended SAA levels (NRC, 2012) and maximum protein deposition was only reached when SAA were supplied at 20% higher levels than currently recommended by NRC (SAA:Lys ratio of 0.75; Kim et al., 2012b). These recent studies clearly showed that a higher SAA is required to attenuate the performance loss induced by immune system activation. Similarly, in a LPS-injected weaner pig model, dietary supplementation of 500 mg/kg N-acetylcysteine, a precursor of L-cysteine, attenuated LPS-induced intestinal damage and tight junction protein gene expression (Hou et al., 2012), production of TNF-α, IL-6 and PGE₂, and TLR4 mRNA expression in the jejunal and ileal mucosae (Hou et al., 2012). These immunomodulative effects of SAA and N-acetylcysteine are most likely through the modulation of reactive oxygen and nitrogen species produced during inflammation and systemic response (Metayer et al., 2008; Hou et al., 2013).

**Dietary n-3 and n-9 polyunsaturated fatty acids**

Metabolism of omega-3 (n-3) fatty acids and impacts of their metabolites on health and systemic response was reviewed in depth by several authors (James et al., 2000; Palmquist, 2009; Lenihan-Geels et al., 2013). The anti-inflammatory effects of n-3 fatty acids are believed to be mediated by increased production of anti-inflammatory eicosanoids in relation to production of n-6 fatty acid-derived pro-inflammatory eicosanoids in the event of a systemic immune response. Presence of n-3 fatty acids in membrane-bound phospholipids compete for the enzymes that are involved in conversion of n-6 fatty acids to pro-inflammatory eicosanoid mediators, such as series 2 prostaglandins and series 4 leukotrienes, and therefore have the ability to reduce production of pro-inflammatory eicosanoids (Wall et al., 2010) (see Figure 3). Such competition and increased production of anti-inflammatory eicosanoids, such as series-3 prostaglandins and series-5 leukotrienes from the membrane-bound n-3 fatty acids in the event of a systemic response, is known to significantly reduce development, severity and duration of the immune response (James et al., 2000; Goddeeris et al., 2002; Palmquist, 2009; Wall et al., 2010; Lenihan-Geels et al., 2013). Membrane-bound n-3 fatty acids also act as substrates for cyclooxygenase and lipoxygenase enzymes that facilitate production of PGE₂ and LTB₄ from metabolism of arachidonic acid (Prescott et al., 2007; Wall et al., 2010). The n-3 fatty acid-driven competitive reduction in the quantity of pro-inflammatory eicosanoids eventually reduce expression of pro-inflammatory cytokines such as TNF-α and IL-1β (Caughey et al., 1996; Chytilova et al., 2013), which are mediated by down-regulation of transcription factors such as nuclear factor-kappaB (NF-κB) and PPARs via inhibition of reactive oxygen species (Anderle et al., 2004; Calder, 2006; Liu et al., 2012; van den Elsen et al., 2013).

There is evidence that supplementation of dietary n-9 fatty acid (oleic acid) also down-regulates production of pro-inflammatory eicosanoids in rats and humans (Stenson et al., 1984; Cleland et al., 1994; James et al., 2000). Eicosatrienoic acid acts as a substrate for 5-lipoxygenase and produces LTA₃ which is a strong inhibitor for the enzyme LTA₄ hydrolase, that is needed for LTB₄ production (Evans et al., 1985; Jakschik et al., 1983; James et al., 2000). The n-6:n-3 fatty acid ratio of 4:1 was suggested as a guideline for optimal immune function in human nutrition (Simopoulos, 2004), while pig diets typically contain a ratio greater than 10:1. Wilkinson et al. (2011) demonstrated that weaner pigs fed an n-6 fatty acid-rich diet were 7 kg lighter at 4 weeks after weaning compared with weaner pigs fed an n-3 fatty acid-rich diet. In a subsequent experiment conducted in a commercial farm, pigs fed an n-6-rich diet were 3 kg lighter and had significantly increased mortality (33% vs. 0.4%) compared with pigs fed an n-3-rich diet at 4 weeks after weaning. In a rat intestinal colitis model, feeding diets containing n-3 and n-9 fatty acids significantly reduced histological damage in the colon compared with rats fed a diet containing n-6 fatty acids (Jacobson et al., 2005). There is evidence that n-3 fatty acids significantly improve intestinal barrier function by increasing cytosolic tight junction protein ZO-1 mRNA expression and paracellular permeability in an in vitro epithelial monolayer model (Li et al., 2008), by increasing extracellular tight junction protein occludin mRNA gene expression and decreasing PPARγ gene expression in the rat ileal mucosa (Wang et al., 2012), and by attenuating IL-4 mediated trans- and para-cellular permeability in an in vitro model (Willemsen et al., 2008).
Boron

Boron is a naturally occurring non-metallic mineral that does not accumulate in the tissue, and is widely used for medical purposes due to its antibacterial properties and ability to reduce heavy metal toxicity (Turkez et al., 2012). In the body, most boron (>96%) exists as boric acid and reacts with molecules with hydroxyl groups to form boron ester. Therefore, boric acid promptly forms complexes with metabolically important sugars such as ribose, which is a component of adenosine (Nielsen, 2009; Nielsen and Meacham, 2011). This chemical characteristic allows boron to react with signaling molecules containing adenosine. For example, Nielsen (2009) demonstrated that boron-deprived rats (0 versus 3 mg boron/kg diet) showed significantly reduced plasma S-adenosyl-methionine and S-adenosyl-homocysteine, and increased circulating homocysteine. Reduced S-adenosyl-methionine is reported in many human diseases such as arthritis, cancer and impaired brain function because S-adenosyl-methionine has a central role for methylation of neurotransmitters, DNA, RNA, proteins phospholipids and hormones (Nielsen, 2009).

Apart from this, dietary boron is known to inhibit both PGE₂ and LTB₄ production by inhibiting cyclooxygenase and lipoygenase activity in leukocytes in rats (Rajendran et al., 1994, Hunt and Idso, 1999, Nielsen et al., 2007) through a NF-κB regulated pathway (Durick et al., 2004). Hunt and Idso (1999) suggested that dietary boron down-regulates leukocyte 6-phosphoglucuronate dehydrogenase and reduces reactive oxygen species produced by neutrophils for local phagocytosis, but exacerbates the inflammatory response in excess (Hunt and Idso, 1999). This notion was supported by a recent rat study (Ince et al., 2010) where supplementation of 100 mg boron/kg as either boric acid or borax significantly decreased an oxidative stress/lipid peroxidation bio-marker malondialdehyde and increased glutathione content in the blood. Boron is also known to inhibit the activity of serine proteases such as tryptase, the proteolytic enzymes released by activated mast cells and leukocytes which degrade structural proteins of pathogens but also degrade structural proteins of the host cells (Hunt, 2003). In addition, a recent in vitro study demonstrated that calcium fructoborate decreased IL-1β, IL-6 and nitric oxide production by LPS-stimulated murine macrophages (Scorei et al., 2010).

Only a few studies have been conducted in pigs and reported the beneficial effects of boron on performance and immune function, although no pig study has specifically measured the effects of boron on PGE₂ and LTB₄ production. Armstrong and Spears (2003) fed either a control diet or a diet supplemented with 5 mg boron/kg as sodium borate and found a significant increase in average daily gain due mainly to increased feed intake during 49 d after weaning (0.47 versus 0.36 kg) and also during the subsequent
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grower phase (next 79 d, 1.00 versus 0.85 kg). More interestingly, this particular study tested a local inflammatory response by measuring skinfold swelling after intra-dermal injection of phytohemagglutin (150 μg) at 120 d of age and found that pigs fed a 5 mg boron diet significantly reduced skinfold swelling. Consistent improvements in daily gain, feed intake and local inflammation response were repeatedly reported in pigs fed a 5 mg boron diet (Armstrong et al., 2001; Armstrong and Spears, 2001; Armstrong et al., 2002). Given that the eicosanoid mediators (PGE2 and LTB4) induce anorexia which is partly responsible for decreased growth in subclinical and clinical infection, the consistent improvement in feed intake and daily gain in pigs fed a 5 mg boron diet may advocate that boron reduced in vivo production of eicosanoids. A dose-response study of boron on production of eicosanoid mediators in immune system compromised pigs is required to clarify the immunomodulatory role of boron in pigs.

**Probiotics, prebiotics, synbiotics and role of non-starch polysaccharide-degrading enzymes**

The presence of commensal bacteria in the intestinal tract improves intestinal barrier function through diverse mechanisms. The importance of commensal bacteria for intestinal barrier function was clearly demonstrated in many studies and one example is a mouse study where a 24-h water medication with streptomycin 2 d prior to oral infection with an enterohaemorrhagic strain of *E. coli* (O157:H7) significantly increased susceptibility to *E. coli* infection compared with mice without antibiotic pre-treatment (Roxas et al., 2010). In this study it was evident that mucosa-bound *E. coli* O157:H7 was significantly increased in the ileum, caecum and colon of the streptomycin (5g/L) pre-treated mice compared with the control mice. Moreover, mucosa-bound *E. coli* O157:H7 was detected at 10^7 CFU/g tissue in the streptomycin pre-treated mice at day 10 after infection, while no mucosa-bound *E. coli* was detected in the control mice. Therefore, both severity and duration of infection were increased by removing commensal bacteria (24 h water medication) in the intestinal tract prior to an event of infection. This result showed not only the importance of commensal bacteria for intestinal barrier function but also the danger of intermittent medication which is frequently used for veterinary intervention in the pig industry.

A healthier commensal microbial composition in the intestinal tract may be manipulated by supplementation of probiotics, prebiotics and (or) synbiotics. The mechanisms for how probiotics manipulate the intestinal barrier function are well summarized by several authors (e.g., Mennigen and Bruewer, 2009; Ng et al., 2009; Ohland and MacNaughton, 2010; Kenny et al., 2011). First, some probiotics such as *Lactobacillus spp.* can directly manipulate intestinal barrier function by increasing mucus gene expression and secretion by goblet cells (Mack et al., 2003; Kim et al., 2008), by amplifying β-defensin expression in the enterocytes (Schlee et al., 2008), and by enhancing tight junction protein gene expression (Resta-Lenert and Barrett, 2003; Ulluwishewa, 2011). Second, probiotics such as *Bifidobacterium spp.* stimulate secretory IgA production in the lamina propria, which is secreted to the mucus layer and binds pathogens and antigens (Shu and Gill, 2001). Third, probiotics reduce pathogen proliferation and attachments by reducing intestinal pH (Ogawa et al., 2001) and by competitive exclusion (Sherman et al., 2005; Johnson-Henry et al., 2008), respectively. In addition, a recent transgenic mice study that impairs apolipoprotein E metabolism as a metabolic disease model, showed that supplementation of probiotics such as *L. rhamnosus* GG and *Propionibacterium freudenreichii* spp. *Shermanii JS* to the transgenic mice induced an inflammatory response by feeding a high-fat diet significantly decreased the number of mast cells in the colonic mucosa (see Figure 4). This report is consistent with previous findings in pigs that some probiotics have the ability to modulate systemic response such as suppressing pro-inflammatory cytokine production (Walsh et al., 2008; Zhang et al., 2010; Chytirova et al., 2013).

Prebiotics are non-digestible feed ingredients that can stimulate growth of beneficial bacteria, and synbiotics are combined supplementation of probiotics and prebiotics (Gibson and Roberfroid, 1995; Gourbeyre et al., 2011). Synbiotics are expected to synergistically build healthier commensal microbial composition compared with individual supplementation of probiotics and prebiotics, if the correct combination of prebiotics and probiotics are used (Schrezenmeir and de Vreese, 2001). Supplementation of oligofructose with a mixture of probiotics (*Lactobacillus spp.*, *Bacillus subtilis*, *Saccharomyces cerevisiae*) to weaned pigs increased bifidobacteria in the ileum and colon and decreased coliform bacteria in the colon (Shim et al., 2005). More recently, Krause et al. (2010) supplemented diets with non-pathogenic *E. coli* and raw potato starch as synbiotics to weaned pigs experimentally infected with a pathogenic *E. coli* K88. Supplementation of synbiotics synergistically improved daily gain, feed intake and faecal score compared with individual supplementation of probiotics or prebiotics. Terminal restriction fragment length polymorphism (T-RFLP) analysis of 16S rRNA genes showed that supplementation of synbiotics synergistically decreased abundance of *Clostridia* in the ileal digesta and synergistically increased microbial richness and diversity in the colon compared with individual supplementation of either probiotics or prebiotics.
Figure 4. Effect of probiotics on mast cell numbers in the proximal colon of transgenic mice that impairs apolipoprotein E metabolism as a metabolic disease model, which increased dietary fat-induced inflammatory responses. Mice were euthanised and tissue samples harvested at d 28 after feeding a high fat diet. GG: Lactobacillus rhamnosus GG. PJS: Propionibacterium freudenreichii spp. Shermanii JS. Fenofibrate is an activator of PPARα with established anti-inflammatory potency and also lipid-modulating capacity (from Oksaharju et al., 2013).

Given the synergistic effect of synbiotics, it is feasible that exogenous non-starch polysaccharide (NSP)-degrading enzymes can be strategically used to produce prebiotics (smaller molecular weight oligosaccharides) in vivo by reducing the molecular weight of the large NSP molecules originated from plant-based feed ingredients in pig diets. An in vitro study showed that addition of a NSP-degrading enzyme at a low dose solubilises insoluble NSP and increase soluble NSP content, while at higher dose rates the enzyme further reduced the solubilised NSP (Castanon et al., 1997). In a recent review, Kiarie et al. (2013) provided evidence that use of xylanase in a wheat-based broiler diet increased short-chain xylo-oligomers in the caecum and increased specific bacteria such as lactobacillus in pigs. Therefore, a low dose of arabinoxylanase in wheat/rye-based diets or β-glucanase in barley/oat-based diets in combination with suitable probiotics may need to be tested as synbiotics to improve intestinal health and hence eventually minimise systemic response in the commercial herds where pigs are consistently exposed to chronic subclinical infection. However, information as to the correct dosage and type of enzyme depends on major cereal grains and oilseed meals that maximise prebiotic production in vivo needs to be explored in detail.

Antioxidants

Other nutrients that have the ability to reduce PGE₂ and LTB₄ biosynthesis are the extracellular antioxidant selenium (Werz and Steinhilber, 1996; Hwang et al., 2006; Vunta et al., 2008) and the intracellular antioxidant vitamin E (Sakamoto et al., 1991; Wu et al., 1998; Venkatraman and Chu, 1999; Reiter et al., 2007; Jiang et al., 2011).

Selenium in the form of selenoproteins plays an important role as a major antioxidant to alleviate the cell damage from reactive oxygen species, especially in macrophages (Vunta et al., 2008). The 5-lipoxygenase enzyme is activated when lipid peroxidation is increased (Riendeau et al., 1989; Imai et al., 1998) and therefore the cellular redox status is an important factor for activation of lipoxygenase in the cell wall (Bryant et al., 1982). An in vitro study with rat granulocytes showed that selenium deficiency reduced fatty acid hydroperoxides and increased cellular 5-lipoxygenase activity (Weitzel and Wendel, 1993). In a human B-lymphocyte study, where cellular leukotriene synthesis was upregulated with transforming growth factor (TGF-β), selenium-dependent peroxidases suppressed cellular 5-lipoxygenase activity (Werz and Steinhilber, 1996). Moreover, selenium decreased COX-2 gene expression in the colon cancer cells (Hwang et al., 2006) and in macrophages, where subsequent reduction in TNF-α was reported (Vunta et al., 2008).

Vitamin E deficiency in pigs is known to decrease markers of cell-mediated immunity such as impaired lymphocyte proliferation (Lessard et al., 1991). Vitamin E supplementation is also known to increase humoral immune responses. For example, Ellis and Vorhies (1976) reported that, compared with a control diet (without vitamin E supplementation with approximately 20 IU/kg in the control diet),
additional vitamin E supplementation at 20 and 100 IU/kg increased two- and three-fold the levels of anti E. coli serum antibody in pigs challenged with an intramuscular injection of E. coli bacterium (0.5 mL of 10^9 cfu/mL, serotype O149:K88, 91:H19). In a broiler study, supplementation of 300 IU/kg vitamin E in an E. coli-challenged group (post-thoracic air sacs, 1.5 mL of 5 x 10^7/mL) reduced mortality from 40% to 5% (Tengerdy and Nockels, 1975). Therefore, a low vitamin E status at weaning can be a predisposing factor for viral (Beck, 2007) and bacterial diseases (Ellis and Vorhies, 1976), and bacterial infection such as E. coli further reduces body vitamin E reserves (Lauridsen et al., 2011). The protective effect of vitamin E has been reported to be associated with inhibition of the biosynthesis of PGE_2 by antagonising the lipid peroxidation of arachidonic acid and limiting the entry of precursor into PGE_2 production (Likoff et al., 1981).

Vitamin E acts as an intracellular antioxidant and is an important essential nutrient for maintenance of immune function in pigs. In an early mice study, it was demonstrated that the mice fed 500 ppm of synthetic vitamin E (dl-α-tocopheryl acetate) reduced the age-related PGE_2 increase in spleen homogenate (Meydani et al., 1986; Wu et al., 1998). In another rat study, α-tocopherol decreased PGE_2 production in the peritoneal macrophage stimulated with phorbol myristate acetate (Sakamoto et al., 1990). However, in a later study, Jiang et al. (2000) reported that γ-tocopherol rather than α-tocopherol is more potent in reducing PGE_2 synthesis by inhibiting COX-2 activity but not by affecting COX-2 protein gene expression in LPS-stimulated macrophages and epithelial cells. Subsequent studies showed that γ-tocopherol, δ-tocopherol and long-chain carboxychromans (metabolites of vitamin E) were potent inhibitors of COX-2 and 5-lipoxygenase and decreased production of PGE_2 and LTB_4 (Jiang and Ames, 2003; Jiang et al., 2008; Jiang et al., 2011). Research to investigate the effect of different types of Vit E on production of eicosanoid mediators in immune system-activated pigs have not been conducted and warrant future study to elucidate the role of vitamin E on immune function of pigs.

Synergistic effects of vitamin E, selenium, n-3 fatty acids and NSAIDs such as aspirin as a COX inhibitor on reducing production of eicosanoid mediators have been reported. An early chicken study showed a synergistic reduction in mortality and PGE_2 production in the bursa when birds provided with both 300 mg/kg vitamin E and 50 mg aspirin/kg body weight in combination compared with individual supplementation of vitamin E or aspirin (Likoff et al., 1981). In a recent rat study the carrageenan-induced inflammation model was used, and Jiang et al. (2009) demonstrated that a combination of γ-tocopherol (33 mg/kg) and aspirin (150 mg/kg) inhibited PGE_2 production by 70% at the inflammation site while a combination of α-tocopherol and aspirin did not show any beneficial effect. Moreover, γ-tocopherol but not α-tocopherol reduced aspirin-induced side effects such as reductions in feed intake and gastric lesion score. Supplementation of vitamin E and selenium in combination synergistically increased antibody titres in antigen-challenged weaner pigs (Peplowski et al., 1981). In a rat oesophageal squamous cell carcinoma model, the combined supplementation of vitamin E and selenium decreased PGE_2 and LTB_4 production and gene expression of COX-2 and 5-lipoxygenase in the esophagus (Yang et al., 2011). In a rat study using the rheumatoid arthritis model, Venkatraman and Chu (1999) reported that combined supplementation of n-3 fatty acid and vitamin E synergistically decreased PGE_2 and LTB_4 contents in the serum.

Conclusions

Pigs in a commercial environment are consistently exposed to (sub) clinical infections and stressors, and systemic responses to such challenges significantly reduce the growth potential of modern pigs. The systemic immune response influences performance of pigs in two distinctive routes: alteration of nutrient partitioning and neurological response to infection such as anorexia. The role of nutrition for a multi-angle approach to tackle both routes of the systemic response was reviewed. Supplementation of selected amino acids that are extensively used during the systemic response to counteract the altered nutrient partitioning, and supplementation of nutrients that reduce production of eicosanoid mediators that in turn provokes a neurological infection response, were suggested as possible solutions. The majority of the evidence provided is largely based on rat infection models or in vitro studies, therefore further pig research for individual and synergistic effects of the suggested nutrients on production of eicosanoid mediators is required to establish robust and cost-effective nutritional strategies for efficient growth of pigs in a commercial production system.

References


