ABSTRACT: Endotoxin, also referred to as lipopolysaccharide (LPS), can stimulate localized or systemic inflammation via the activation of pattern recognition receptors. Additionally, endotoxin and inflammation can regulate intestinal epithelial function by altering integrity, nutrient transport, and utilization. The gastrointestinal tract is a large reservoir of both gram-positive and gram-negative bacteria, of which the gram-negative bacteria serve as a source of endotoxin. Luminal endotoxin can enter circulation via two routes: 1) nonspecific paracellular transport through epithelial cell tight junctions, and 2) transcellular transport through lipid raft membrane domains involving receptor-mediated endocytosis. Paracellular transport of endotoxin occurs through dissociation of tight junction protein complexes resulting in reduced intestinal barrier integrity, which can be a result of enteric disease, inflammation, or environmental and metabolic stress. Transcellular transport, via specialized membrane regions rich in glycolipids, sphingolipids, cholesterol, and saturated fatty acids, is a result of raft recruitment of endotoxin-related signaling proteins leading to endotoxin signaling and endocytosis. Both transport routes and sensitivity to endotoxin may be altered by diet and environmental and metabolic stresses. Intestinal-derived endotoxin and inflammation result in suppressed appetite, activation of the immune system, and partitioning of energy and nutrients away from growth toward supporting the immune system requirements. In livestock, this leads to the suppression of growth, particularly suppression of lean tissue accretion. In this paper, we summarize the evidence that intestinal transport of endotoxin and the subsequent inflammation leads to decrease in the production performance of agricultural animals and we present an overview of endotoxin detoxification mechanisms in livestock.

Keywords: endotoxin, inflammation, intestine

INTRODUCTION

Growth performance of agricultural animals in commercial settings is affected by various physical, social, and microbial factors that may predispose animals to physiological or immunological stresses (Hollek et al., 1998). Among the stressors that can attenuate the growth performance of animals are viruses, live bacteria, and dead bacteria that contain cell wall compounds such as lipopolysaccharide (LPS) and peptidoglycans (Schinckel et al., 1995; Smith, 1998). Specific to the focus of this review, we discuss LPS, otherwise referred to as endotoxin, the cell wall component of gram-negative bacteria that is a potent immune stimulator in livestock (Webel et al., 1997; Kimball et al., 2003). Importantly, the gastrointestinal tract (GIT) of animals serves both as a major barrier to, and major

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source of, endotoxin (Ravin et al., 1960; Schweinburg and Fine, 1960; Wiznitzer et al., 1960).

Endotoxin in mammals is recognized by various cells expressing the pattern recognition receptor, toll like receptor (TLR) 4, and other proteins including LPS binding protein (LBP), cluster of differentiation 14 (CD14), and myeloid differential protein 2 (MD2). These proteins and receptors are also present in intestinal epithelial cells and have been associated with the transport of luminal endotoxin into circulation (Hornef et al., 2003; Neal et al., 2006). Once in the systemic circulation, endotoxin can be deactivated or detoxified by immune cells, such as macrophages, or Kupffer cells present in the liver or splenic cells or by binding with acute phase proteins (Rutenburg et al., 1967; Satoh et al., 2008; Buttenschoen et al., 2010). However, if there is failure of systemic detection and deactivation, increased circulating concentrations of endotoxin can lead to local and systemic inflammation and, if severe enough, endotoxemia and even death (Zweifach and Janoff, 1965; Rice et al., 2003). The importance of endotoxin to livestock production is that chronic activation of the immune system has been shown to antagonize the growth and performance of animals because nutrients are being partitioned toward production of cytokines, acute phase proteins, and other immune modulators rather than toward the anabolic processes that support milk and muscle synthesis (Johnson, 1997; Spurlock, 1997). Further, endotoxin can lead to various diseases, including colic and laminitis, and endotoxemia is a leading cause of death in equine species (Sykes and Furr, 2005; Werners et al., 2005). Lipopolysaccharide has also been shown to activate the heterophils and upregulate the proinflammatory cytokine and chemokine expression in poultry (Kogut et al., 2005).

Interestingly, transport of endotoxin from the intestine has been shown to be modulated by dietary factors as well as by stressors, including heat stress, systemic disease, and feed restriction or malnutrition (Hall et al., 2001; Cani and Delzenne, 2010). The major dietary factor that appears to modulate the transport of luminal endotoxin is dietary fat. As indicated in the biomedical and human health literature, as the percentage of dietary fat increases, so does the concentration of circulating endotoxin (Erridge et al., 2007; Amar et al., 2008). Supporting this notion, we recently found that feeding young pigs a high fat diet increases serum endotoxin (V. Mani and N. K. Gabler, unpublished data). Further, the form of the lipid ingested may modulate the endotoxin transport, with emulsified lipids increasing endotoxin transport (Mani and Gabler, 2010; Mani et al., 2010; Laugerette et al., 2011). In ruminants, feeding easily digestible carbohydrates and grains has been shown to increase the transport of endotoxin to the peripheral circulation, indicating that carbohydrates also influence endotoxin transport (Khafipour et al., 2009; Zebeli et al., 2011). In addition to dietary nutrients, systemic increases in intestinally derived endotoxin can be attributed to environmental and immunological stressors. Hyperthermia increases intestinal permeability and, presumably, intestinal endotoxin transport (Lambert, 2004, 2008; Pearce et al., 2011). Plasma antibodies to endotoxin are inversely related to growth in malnourished young children and are associated with increased intestinal permeability and systemic immune system activation (Campbell et al., 2003). Further studies are warranted to investigate the relationship between endotoxin and growth in livestock.

**ENDOTOXIN**

Lipopolysaccharide is a glycolipid present in the outer membrane of gram-negative bacterial cell wall. Lipopolysaccharide consists of a hydrophobic domain, lipid A, through which it is inserted into the bacterial cell wall, a core oligosaccharide, and a distal oligosaccharide (Elin and Wolff, 1976; Raetz and Whitfield, 2002). The hydrophobic lipid A domain is the most biologically active portion of the LPS molecule and it is synonymously known as endotoxin because of its toxic nature (Erridge et al., 2002). In a typical Escherichia coli, lipid A contains the following structural properties: 1) the backbone of the lipid A contains diglucosamine, which is phosphorylated at positions 1′ and 4′; 2) two 3-hydroxyymristate molecules are directly attached to each glucosamine; and 3) at positions 2′ and 3′, the hydroxyl groups of the fatty acids are substituted by laurate and myristate and they form an acyloxyacyl bond with the primary fatty acid chains (Figure 1). Diphosphorylated hexaacyl lipid A molecules have been shown to be effective stimulators of the immune system because they have been optimally recognized by the mammalian immune system. Monophosphorylated or dephosphorylated endotoxin molecules have been shown to substantially lose their potency and immune reactivity (Holst et al., 1996; Munford, 2005). Another important characteristic of lipid A is that mostly all the fatty acyl chains are made up of saturated fatty acids (SFA). If the SFA are replaced with unsaturated fatty acids, the endotoxin molecule causes an attenuated immune response and becomes less of an immune stimulant (Munford and Hall, 1986; Kitchens et al., 1992).

Endotoxin can enter systemic circulation from live bacteria, leading to septicemia, or as cell wall components of dead bacteria. Either way, if the amounts are too great, they can ultimately antagonize anabolic growth (Kimball et al., 2003; Orellana et al., 2007) or lead to septic shock and death (Moore and Morris, 1992). Endotoxin is released during bacterial death and during growth and division, making it a ubiquitous contaminant (Petsch and Anspach, 2000; Yaron et al., 2000). The biological activity of the endotoxin is measured in endotoxin units (EU). For example, 100 pg of endotoxin is considered to have 1 EU,
Mani et al. 1454
and 10 EU is equivalent to 1 ng of endotoxin. A single gram-negative bacterium contains approximately $10^{-15}$ g of LPS, and $10^5$ bacteria can generate 1 EU. It has been shown that a single *E. coli* contains approximately $10^6$ lipid A residues ([Raetz et al., 1991](#)). Furthermore, the size of the individual endotoxin molecules varies between 10 and 20 kDa in monomeric form and, because of the amphiphilic nature, they can arrange themselves into large micellar structure achieving 1,000 kDa.

**GASTROINTESTINAL FUNCTION**

The lumen of the GIT is considered a space outside the body because of its continuity with the external environment. It has the arduous task of absorbing the nutrients that are essential for the organism while preventing the absorption of substances that are not needed and are harmful to the system. The GIT primarily serves two important functions: absorbing nutrients from the lumen and forming a barrier between the luminal contents and systemic circulation. Primarily, the intestines aid in the digestion and absorption of proteins, carbohydrates, lipids, vitamins, minerals, and water. A single layer of intestinal epithelial cells (IEC), which line the intestine, selectively absorbs most of the nutrients needed through active and passive processes with the help of specific transport or carrier proteins. For example, glucose and fructose are absorbed through Na-dependent glucose transporter 1 and glucose transporter 5, respectively. Water is absorbed through aquaporin

*Figure 1. Simplified structure of lipopolysaccharide (LPS) from gram-negative bacteria such as *Escherichia coli*. Lipopolysaccharide contains a distal “O” polysaccharide region, a core polysaccharide region divided into outer and inner core and an interior lipid A component through which LPS is inserted into the cell membrane. “O” polysaccharide region is highly variable and contains approximately 10 to 25 repeated units and is made up of common hexose (Hex) sugars. Outer core polysaccharide contains common hexose sugars such as glucose (Glc) and galactose (Gal), whereas inner core polysaccharide contains unusual sugar such as 3-deoxy-D-manno-octulosonic acid (Kdo). Lipid A structure is explained in the text. Arrows labeled alkaline phosphatase (AP) and acyloxyacyl hydrolase (AOAH) indicate the cleavage points where these enzymes cleave the phosphate and secondary fatty acyl chains, respectively. GlcN = N-acetyl glucosamine; Hep = Heptose.*
receptors, and amino acids and di- and tripeptides are absorbed through numerous transporter proteins located on the apical and basolateral membranes. Additionally, the GIT serves as a major excretory organ that helps in waste products, including excessive nutrients and toxic substances secreted by the biliary system. This task becomes more difficult because only a single layer of IEC serves as a barrier. The IEC form a membrane that acts as a selective permeability barrier, which can selectively allow substances from the lumen.

The epithelial or intestinal integrity is critical for maintaining a physical barrier between the intestinal lumen and the body. This is dependent largely on the junction complexes connecting enterocytes together and is achieved via a well-organized intercellular array of tight junctions, adhesion junctions, and desmosomes surrounding the apical region of epithelial cells. Cell-to-cell adhesion and tight junctions are regulated by the membrane-spanning proteins claudin, occludin, zonula occludens (ZO) 1 and 2, and cingulin (Oswald, 2006; Turner, 2006). Additionally, adhesion junction proteins, such as E-cadherin, contribute to gut integrity. Tight junctions are the most apical junctions between 2 epithelial cells that are formed by claudin and occludin family protein strands along with other protein complexes (Denker and Nigam, 1998; Chiba et al., 2008). It is becoming clear that different claudin isoforms participate in intestinal barrier function. Together, claudin and occludin proteins are attached to actin cytoskeleton through other proteins, such as ZO-1 and junction adhesion molecules (Nusrat et al., 2007; Nusrat et al., 2000; Turner, 2006). It was initially thought that tight junctions form a physical barrier without any cellular regulation, but recent research indicates that tight junction proteins are very well regulated; intracellular translocation of tight junction proteins from the cell membrane and back occurs regularly during normal cellular processes (Shen et al., 2011).

**ENDOTOXIN SIGNALING AND TRANSPORT**

Innate immune response is the first line of defense against infectious diseases, is mediated by white blood cells such as neutrophils and macrophages, and is thought to be nonspecific (Aderem and Ulevitch, 2000). However, a series of discoveries in the late 1990s proved this theory wrong (Medzhitov et al., 1997). Specific receptors present in immune cells, such as macrophages, dendritic cells, B-cells, and certain types of T-cells, could recognize a particular pattern in the invading microbes; these receptors came to be known as pattern recognition receptors (Medzhitov, 2001; Janeway and Medzhitov, 2002). Moreover, myocytes and adipocytes also express these same pattern recognition receptors (Gabler and Spurlock, 2008). The ‘patterns’ present in the different microbial species are essential for their survival and have come to be known as pathogen-associated molecular patterns, later renamed microbe-associated molecular patterns (MAMP), to include all the microbes, including pathogens and nonpathogens (Ausubel, 2005; Akira et al., 2006). Pattern recognition receptors sense the presence of a variety of molecules from the invading pathogens, as well as commensals, and regulate the immune response by stimulating the secretion of various immune mediators (Brikos and O’Neill, 2008). More recently, pattern recognition receptors have been shown to recognize not only the pathogenic patterns but also commensals, as well as cellular degradation products from the same organism, which are known as damage-associated molecular patterns (Chen and Nunez, 2010; Rosin and Okusa, 2011). The first toll pattern recognition receptor to be identified was TLR4, which recognizes bacterial endotoxin and other proteins, including heat shock proteins (Poltorak et al., 1998). At present, there are 11 human and 13 murine TLR, which recognize different pathogen components, including flagella, peptidoglycan, double-stranded RNA, and DNA (McGettrick and O’Neill, 2010; Moresco et al., 2011).

The presence of endotoxin is not sensed by TLR4 alone. Endotoxin is usually present as an aggregate bound to other endotoxin molecules on which LBP acts and separates a monomer, which is then presented to CD14. The CD14 receptor is present in two forms: membrane bound or soluble. The CD14 protein does not have an intracellular domain, so it associates with TLR4, which has a toll-IL 1 receptor intracellular domain (Beutler, 2000; Triantafilou and Triantafilou, 2002). Toll-like receptor 4 then dimerizes and binds with myeloid differential protein 2, which transmits the signal through the toll-IL 1 receptor intracellular domain through two pathways. One is a myeloid differentiation factor 88-dependent pathway and the other is a myeloid differentiation factor 88-independent pathway. The first pathway leads to translocation of nuclear factor kappa β to the nucleus and the initiation of gene transcription of inflammatory mediators. Alternately, the independent pathway leads to the activation of interferon regulatory factor 3 as well as nuclear factor kappa β (Verstrepen et al., 2008; Coll and O’Neill, 2010). Basically, both pathways lead to the secretion and stimulation of proinflammatory cytokines and other immune mediators. The signaling is quenched by endocytosis of TLR4, along with LPS, to an endosome where it is then degraded (Saitoh, 2009).

Current research indicates that apart from the signaling proteins, lipid rafts are essential for the TLR4 signaling and transport to occur (Pfeiffer et al., 2001; Triantafilou et al., 2002, 2004; Olsson and Sundler, 2006). Lipid rafts are specialized membrane domains, which are rich in SFA, cholesterol, and sphingolipids (Brown
These pathogens and MAMP can stimulate the localized environment where the bacteria can be ingested along with feed and water or through respiration (Spaan et al., 2006). The other source is the commensal bacteria in the GIT, which is a rich source of gram-negative organisms (Wiznitzer et al., 1960; Ley et al., 2006). The bacterial population is very scarce in the stomach because of the acidic environment, but the numbers increase exponentially down the intestinal tract from duodenum to colon (Tlaskalová-Hogenová et al., 2004; Magalhaes et al., 2007). The main entry point for pathogenic bacteria, endotoxin, mycotoxin, and other pathogens is via the digestive tract. Thus, the intestines form a major physical barrier to prevent pathogens and toxic compounds from entering the mucosa and circulation and then activating the immune system.

The luminal transport of endotoxin to the systemic circulation is not fully understood, but 2 primary routes exist. The first route is through paracellular transport, where the transport of endotoxin occurs through tight junctions formed between 2 intestinal epithelial cells (Drewe et al., 2001; Hietbrink et al., 2009). Various factors have been shown to regulate the permeability of the intestinal tight junction barrier (Shen et al., 2011). When animals are under stress or have intestinal inflammation, small quantities of luminal contents, endotoxin, and pathogens may enter the epithelium and circulation through the tight junctions. These pathogens and MAMP can stimulate the localized secretion of proinflammatory cytokines, including tumor necrosis factor (TNF)-α and IL-1β from immune and intestinal epithelial cells. Consequently, these inflammatory and stress responses may cause the phosphorylation of myosin light chain by myosin light chain kinase, which results in the contraction and opening of the intestinal epithelial tight junctions and increases intestinal permeability (Turner et al., 1997; Moriez et al., 2005; Chen et al., 2006; Turner, 2009).

Disruption of tight junctions and increased paracellular permeability by oxidative stress has been demonstrated in IEC. Treating Caco-2 intestinal epithelial-like cells with the oxidant hydrogen peroxide, increases barrier permeability and leads to a redistribution of ZO-1 and occludin (Sheth et al., 2009). Interestingly, Caco-2 cell monolayers treated with endotoxin increases lipid peroxidation and paracellular permeability (Courtois et al., 2003). The increased permeability can be reversed by treatment with the antioxidant butylated hydroxytoluene. This indicates that endotoxin itself can decrease intestinal barrier function by a mechanism that is mediated by oxidative stress. Further indicating a link between redox status and intestinal barrier function is the finding that treating Caco-2 cells with bile acid (i.e., cholic acid) increases paracellular permeability by increasing reactive oxygen species (Araki et al., 2005). Blocking the increase in reactive oxygen species with the antioxidant n-acetyl cysteine prevents the decrease in transepithelial electrical resistance (TER) in bile acid-treated IEC. Bile acid treatment leads to redistribution of ZO-1 and occludin and the increased permeability can be reversed by 1-(5-iodonaphthalene-1-sulfonyl)-1H-hexahydro-1,4-diazepine hydrochloride, a myosin light chain kinase inhibitor, indicating a linkage between cellular redox status and tight junctions.

Under normal physiological conditions, tight junction barrier integrity remains intact and luminal contents and transport of molecules across the tight junctions is very well regulated (Edelblum and Turner, 2009). Nevertheless, metabolic stress and environmental stresses, such as heat stress, have been reported to cause increased intestinal permeability or “leaky gut” (Lambert et al., 2002; Lambert, 2004; Singleton and Wischmeyer, 2006). However, the pathways through which tight junction proteins are regulated by these conditions are not fully characterized.

Intestinal and systemic diseases are associated with leaky epithelial barrier and increased intestinal permeability to endotoxin. The TER of cell monolayers or intestinal epithelial membranes is a good indicator of the degree of tight junction organization and gut integrity. Pigs challenged with endotoxin showed altered intestinal TER compared with their controls, indicating that changes have occurred in intestinal integrity and junction organization (Albin et al., 2007). Furthermore, treatment with the n-3 fatty acids, eicosapentaenoic acid and docosahexaenoic acid, has effectively been shown to prevent reduced TER induced by the proinflammatory cytokines, interferon-γ, and TNF-α and prevent the redistribution of occludin and ZO-1 (Li et al., 2008). Also, docosahexaenoic acid treatment of Caco-2 monolayers has been shown to increase paracellular permeability via the intracellular redistribution of the tight junction proteins (Roig-Pérez et al., 2004).

The second route of intestinal endotoxin and bacteria transport is via transcellular transport occurring through the epithelial cells (Tomita et al., 2004; Neal et al., 2006). Further, evidence indicates that lipid rafts are required for the recruitment of TLR4 and that receptor-mediated endocytosis is a key mechanism of transcellular transport of bacteria and endotoxin in many cell types.
(Triantafilou et al., 2002; Ancuta et al., 2008; Chassin et al., 2008). Initially, it was thought that IEC did not have the necessary receptors to recognize the innate immune ligands such as MAMP and damage-associated molecular patterns. However, research over the past 10 yr has discovered that IEC do play a major role in the recognition of pathogens and endotoxin, and IEC express specific receptors, including TLR, nucleotide oligomerization domain receptors, and retinoic acid-inducible gene 1 protein (RIG)-I-like receptors (Cario et al., 2000; Cario, 2005; Santaolalla et al., 2011). Almost all of the TLR, which are present in the immune cells, have been described in human IEC (Abreu, 2010a). The confounding issue regarding the presence of TLR in IEC is that they are expressed on the apical side of the membrane; however, they are not believed to be continually activated by the presence of luminal endotoxin. Research with IEC has shown that TLR4 is present on the apical and basolateral membranes as well as within the golgi apparatus (Hornef et al., 2003; Cario and Podolsky, 2006; Abreu, 2010b). However, the overall consensus regarding the TLR4 location and expression indicates that IEC have a hyporesponsiveness toward endotoxin and that the location of TLR4 within the cell may be a major contributing factor for the hyporesponsiveness (Vamadevan, 2010).

Both paracellular and transcellular routes of transport are two important ways through which most of the endotoxin enters systemic circulation from the gut. The gut is the first line of defense against endotoxin and, if compromised via nutrition, stress or metabolic state, endotoxin transport can increase (Suganuma et al., 2002; Clark et al., 2009; Liu et al., 2009). A greater understanding of gut endotoxin transport (Figure 2) will allow for the development of nutritional and pharmacological mitigation strategies to avert the negative effects of endotoxin and improve production efficiencies in livestock.

**ENDOTOXIN DETOXIFICATION**

After crossing the intestinal barrier, endotoxin is transported by both lymph and blood; however, most of the endotoxin is transported to the liver through the portal vein where a major portion of the endotoxin detoxification process occurs (Olofsson et al., 1986; Van Leeuwen et al., 1994; Lemaire et al., 1999). If the amount of endotoxin entering the gastrointestinal tract overwhelms the detoxification capacity of the liver, endotoxemia ensues (Olofsson et al., 1985). Mammals have developed an elaborate system to tolerate and detoxify endotoxin either at the mucosal surface or in systemic circulation. Endotoxin tolerance can also occur by the downregulation of proteins that participate in endotoxin signaling and the innate immune response (Fan and Cook, 2004). Bile plays an important role in detoxifying the endotoxin because of the detergent action of bile salts in the intestine lumen. Furthermore, endotoxin detection by hepatocytes and Kupffer cells, active and inactive forms of endotoxin may be transferred to the bile and excreted into the lumen (Maitra et al., 1981; Lóránd, 2004). Approximately 7% of the absorbed endotoxin is excreted through bile. Munford (2005) describes 4 mechanisms through which endotoxin may be neutralized. First, there are molecules that bind endotoxin and prevent it from engaging TLR4. Second, there are enzymes that degrade lipid A to decrease its activity. Third, endotoxin can be deactivated after its uptake by the liver. Fourth, there are target cell adaptations that modify the response to endotoxin. Moreover, reports show that incubation of endotoxin with plasma makes it less pyrogenic and less inflammatory (Rall et al., 1957; Rudbach and Johnson, 1964; Ulevitch and Johnston, 1978). Specific plasma proteins are able to bind endotoxin, and this is speculated to aid in the inactivation and detoxification of endotoxin (Rudbach and Johnson, 1966; Johnson et al., 1977; Brade and Brade, 1985). An example of a plasma protein includes serum amyloid A. This acute phase protein has been shown to increase during the acute phase response, binds to endotoxin monomers, and eliminates this toxin via the liver (Coetzee et al., 1986; Emmanuel et al., 2008). Additionally, antibodies such as collectins, along with bactericidal permeability-increasing protein and neutrophil granules, are plasma proteins that bind and neutralize endotoxin (Chaby, 2004; Munford, 2005).

Intestinal chylomicrons, which are involved in transporting the absorbed fatty acids, have been shown to promote the absorption of endotoxin (Ghoshal et al., 2009). However, chylomicrons have been reported to mitigate the toxic effects of endotoxin by binding the endotoxin and promoting its inactivation via contact and the action of bile (Harris et al., 1993; Read et al., 1993). Further, LBP can bind to the chylomicrons and enhance the binding of endotoxin to the chylomicrons, which helps in reducing its bioactivity (Vreugdenhil et al., 2003). Binding of endotoxin to the chylomicron helps in its recognition by low density lipoproteins (LDL) and LDL-associated receptors present in hepatocytes, which promote the endocytosis of endotoxin into the cell and its rapid clearance from circulation (Harris et al., 2002). Presence of apolipoprotein E in the chylomicrons is also protective against endotoxin because it delivers the endotoxin directly to hepatocytes, bypassing Kupffer cells and their proinflammatory cytokine production (Van Oosten et al., 2001). Endotoxin is also found to bind with high density lipoprotein (HDL; Ulevitch et al., 1979). The role of HDL in detoxifying the endotoxin seems to be controversial. It is suggested that HDL aids in sequestering and detoxifying endotoxin but makes it more difficult to clear from circulation (Vreugdenhil et al., 2003; Birjmohun et al., 2007). Further, endotoxin may be trans-
ferred from HDL to LDL with the help of LBP and phospholipid transfer proteins. The transfer of endotoxin to LDL results in dyslipidemia and the loss of the capacity of HDL to bind cholesterol (Levels et al., 2005).

A major detoxification mechanism for endotoxin is by enzyme modification via acyloxyacyl hydrolase (AOAH). This hydrolase enzyme is classified as a lipase and is present in macrophages, dendritic cells, neutrophils, hepatic, liver, and renal cortical tubule cells (Erwin and Munford, 1991). Interestingly, AOAH can be produced by the renal cortical tubule cells where it is secreted into the urine and can deacylate LPS and neutralize endotoxin (Feulner et al., 2004). Acyloxyacyl hydrolase selectively removes the secondary fatty acyl chains attached to the primary chains in the lipid A moiety, producing an LPS structure that is capable of binding MD2/TLR4 but does not initiate the signal or only can be a partial agonist (Lu et al., 2005). It is believed that AOAH has a role in mediating macrophage tolerance to endotoxin because AOAH mRNA abundance is increased in endotoxin-primed and -tolerant macrophages versus endotoxin-naïve macrophages (Mages et al., 2007). When compared with wild-type mice, mice that lack AOAH and are challenged with endotoxin have enlarged livers and sustained hepatic cytokine production, indicating that this enzyme prevents prolonged inflammatory reaction to endotoxin (Shao et al., 2011).

Regarding agriculturally relevant species, AOAH activity is increased during localized inflammation in cattle, and its activity has been localized to neutrophils (McDermott and Fenwick, 1992). The regulation of AOAH by stressors and diet, together with its direct role in intestinal detoxification, warrants further investigation in livestock.

Further evidence that enzyme modification plays a role in endotoxin neutralization and detoxification is supplied by recent reports that intestinal alkaline phosphatase (AP) directly deactivates endotoxin (Bates et al., 2007; Goldberg et al., 2008). Mechanistically, AP deactivates endotoxin by dephosphorylating the diphosphoryl moiety of lipid A, rendering it inactive (Poelstra et al., 1997; Koyama et al., 2002; Munford et al., 2009). Alkaline phosphatase had been shown to inactivate endotoxin in zebra fish (Bates et al., 2007) and its activity is increased in inflamed intestinal tissue (Sánchez de Medina et al., 2004). Also, debate exists regarding how AP dephosphor-
ylates endotoxin, and evidence is limited in livestock as to its role in detoxification. The expression and activity of intestinal AP can be modulated by stress and dietary factors (Lallès, 2010). Dietary lipids regulate the activity of intestinal AP. For example, jejunal AP activity was greater in pigs fed a diet high in saturated fat (i.e., 15% beef tallow) than in pigs fed a diet high in unsaturated fat (i.e., 15% corn oil; Dudley et al., 1994). Another example indicating that AP is regulated by dietary lipids is that cod liver oil rich in n-3 fatty acid has been shown to increase the secretion of intestinal AP (Kaur et al., 2007). Interestingly, this may be explained by the increased expression resolvin-E1, an antiinflammatory n-3 fatty acid lipid mediator, which induces AP activity (Campbell et al., 2010). Furthermore, increased dietary fat consumption reduces intestinal AP activity in obesity-prone rodents (de La Serre et al., 2010). Interestingly, the decrease in ileal AP activity is associated with an increase in plasma endotoxin and increased inflammation as assessed by myeloperoxidase activity (de La Serre et al., 2010).

Mechanistically, the alteration of intestinal AP by dietary lipids may be mediated by proinflammatory cytokines such as IL-1β and TNF-α, which inhibit the induction of AP (Malo et al., 2006). Stress and disease in livestock may decrease intestinal AP via reductions in feed intake (Goldberg et al., 2008; Lallès and David, 2011). It has been observed that weaning pigs at a young age (i.e., 10 d) decreases both the expression and activity of AP in the jejunum (Lackeyram et al., 2010). This same age period near weaning is also associated with decreased feed intake and increased intestinal proinflammatory cytokine expression (Pié et al., 2004), both of which are perhaps responsible for decreased intestinal AP expression and activity that occurs with weaning in pigs. Altogether, dietary factors and stressors likely affect intestinal and systemic inflammation and endotoxin concentrations via alterations in mechanisms of detoxification and neutralization.

**IMPLICATIONS OF INTESTINAL ENDOTOXIN AND INFLAMMATION**

The gastrointestinal tract is the major site of nutrient uptake. The nutrient transport function of the GIT decreases when the intestine is under prolonged immune or metabolic stress. A study looking at absorptive function of the small intestine during endotoxemia showed that Na and Cl ions, as well as glucose absorption, were decreased after 24 h of the challenge (Kanno et al., 1996). Further, marked epithelial inflammation occurs around 6 h after challenge and villous atrophy occurs at 24 h; however, there are signs of recovery after 7 d. It has also been shown that endotoxin challenge results in decreased absorption of various sugars and AA (Meng et al., 2005; Albin et al., 2007; Flinn et al., 2010). One mechanism for this decreased transport might be through the inhibition of Na-dependent system of transport as well as a decrease in the Na+/K+ ATPase activity (Abad et al., 2001; García-Herrera et al., 2003; Amador et al., 2007a). Further, the proinflammatory cytokine TNF-α has been shown to decrease the absorption of galactose (Amador et al., 2007b). Interestingly, intestinal nutrient transport, when challenged with endotoxin, is divergently altered depending on the breed of animal (Albin et al., 2007). Overall, the endotoxin-mediated inhibition of nutrient absorption seems to be manifested by several interrelated signaling cascades, including those involving protein kinase C, protein kinase A, and mitogen-activated protein kinases, as well as proteasomal degradation (Amador et al., 2008; García-Herrera et al., 2008). While the animal itself tries to fight the cause of the stress, the intestine develops a reduced ability to transport nutrients and carry out other functions. The end result is an increased catabolic cascade and degradation of muscle proteins (Webel et al., 1998; Daiven et al., 2008) to support gluconeogenesis and increased whole-body metabolic energy demands.

Because of its strategic position between the luminal microbes and essentially sterile systemic circulation, the intestine needs to possess excellent immune capabilities to defend against any pathogenic attack. Thus, evolutionarily, the intestine developed an extensive immune system network. The gastrointestinal tract can be classified as the largest immune organ in the body (Collins et al., 1998; Fiocchi, 2003). Specific lymph nodes that are part of gut-associated lymphoid tissue are placed in the submucosal layer to defend against any invading pathogens. A variety of mononuclear phagocytes, such as monocytes-macrophages and dendritic cells, are present in the gut-associated lymphoid tissue as well as dispersed throughout the subepithelial connective tissue, the lamina propria. These immune cells, when isolated during intestinal inflammation, display proinflammatory profiles and secrete cytokines such as TNF-α (Bar-On et al., 2011). A typical intestinal inflammatory response progresses through the following steps. A leaky intestinal epithelial barrier allows luminal commensal organism components to enter the submucosa and stimulate the immune system. The dendritic cells, through their pattern recognition receptor, recognize these commensal constituents as pathogen components and initiate the differentiation of T cells and natural killer cells. The immune cells can also be activated by their own pattern recognition receptors. This leads to the secretion of regulatory cytokines by T cells, which in turn stimulate the secretion of TNF-α, IL-1, and IL-6 by macrophages. Natural killer cells also play an active role by secreting cytokines as well as causing tissue damage (Baumgart
and Carding, 2007). The interplay of all these immune cells, endotoxin, and cytokines secretion can augment the inflammatory state of the intestine.

During an inflammatory response, the nutrient partitioning is redirected toward meeting the metabolic requirements of the immune system. Inflammation is associated with increase in body temperature; an increase of 1°C equates to a 13% increase in the basal metabolism (Kluger, 1978). The increase in the concentrations of proinflammatory cytokines such as TNF-α and IL-1β have been shown to decrease feed consumption (Plata-Salamán et al., 1996), rates of BW gain, and efficiency of feed utilization (Evock-Clover et al., 1997; Steiger et al., 1999). Thus, endotoxin-associated inflammation results in an estimated 30% increase in energetic costs and leads to a significant negative nitrogen balance because of protein breakdown and decreased BW gain (Lochmiller and Deerenberg, 2000). Further, multiple immune challenges occurring simultaneously lead to a cumulative reduction in performance (Hanssen et al., 2004).

All the evidence indicates that a significant decrease in feed intake occurs during an immune challenge. Appetite regulation is a complex process, and it occurs mainly through neuronal control through the vagus nerve or through hormonal control via the secretion of leptin, ghrelin, cholecystokinin, and glucagon-like peptide 1. The hypothalamus receives and integrates these signals and brings about the desired effect of altered appetite control (Cummings, 2006; Sartin et al., 2011). The appetite regulation under an immune challenge might occur through either one or both of these mechanisms. During most disease conditions in livestock a reduction in feed intake is accompanied by an increase in metabolic rate, which is significantly different than fasting because during fasting the decrease in feed intake is accompanied by decreased metabolic rate (Sartin et al., 2011). The inflammatory cytokines secreted upon an immune challenge decrease feed intake and nutrient transport by acting on the ST axis (Johnson, 1997, 1998). Tumor necrosis factor-α has been shown to be present in the central nervous system after an immune challenge with endotoxin, which indicates that it could act on the appetite regulatory center directly (Sakumoto et al., 2003). The appetite-stimulating neurotransmitters in the hypothalamus, such as neuropeptide Y and Agouti-related protein, may be reduced or unchanged, whereas appetite-inhibiting neurotransmitters, including proopiomelanocortin and cocaine- and amphetamine-regulated transcript, are increased during immune challenges. During disease stress, the latter may promote α-melanocyte stimulating hormone suppression of appetite via the MC4 receptors to decrease appetite (Sartin et al., 2008, 2011). This mechanism of action results in typical sickness behavior such as decreased appetite and increased energy expenditure (Grossberg et al., 2010).

The other plausible mechanism by which appetite is regulated under an immune challenge and inflammation is that endotoxin and other TLR4 ligands, such as SFA, have been shown to activate the enteroendocrine cells that act as nutrient sensors in the intestine. This leads to the secretion of appetite-regulating peptides, such as cholecystokinin and glucagon-like peptide 1, from the enteroendocrine cells that act on the satiety centers in the hypothalamus and ultimately results in reduced feed intake and nutrient absorption from the intestine (Bogunovic et al., 2007; de Lartigue et al., 2011). Although this has been shown in the cell lines, further research is needed to prove this theory in whole animals.

**SUMMARY AND CONCLUSION**

The literature reviewed herein describes how luminal endotoxin is transported and its effects on gastrointestinal function and animal performance (Figure 2). Additionally, we briefly describe plausible mechanisms of endotoxin detoxification and neutralization. Even at low concentrations, endotoxin is a potent stimulator of proinflammatory cytokine production from various cell types within the body, not just immune-competent cells. The resulting immune activation and associated inflammation makes endotoxin an important factor that is commonly overlooked in livestock production. However, more research is needed to understand how endotoxin is transported into circulation and its effect on metabolism and energetics. Additionally, research describing how stress and nutrition modulate endotoxin transport and clearance in agriculturally relevant species is warranted.

**LITERATURE CITED**


