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Intestinal Alkaline Phosphatase: Potential Roles in Promoting Gut Health in Weanling Piglets and Its Modulation by Feed Additives — A Review

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ABSTRACT: The intestinal environment plays a critical role in maintaining swine health. Many factors such as diet, microbiota, and host intestinal immune response influence the intestinal environment. Intestinal alkaline phosphatase (IAP) is an important apical brush border enzyme that is influenced by these factors. IAP dephosphorylates bacterial lipopolysaccharides (LPS), unmethylated cytosine-guanosine dinucleotides, and flagellin, reducing bacterial toxicity and consequently regulating toll-like receptors (TLRs) activation and inflammation. It also desphosphorylates extracellular nucleotides such as uridine diphosphate and adenosine triphosphate, consequently reducing inflammation, modulating, and preserving the homeostasis of the intestinal microbiota. The apical localization of IAP on the epithelial surface reveals its role on LPS (from luminal bacteria) detoxification. As the expression of IAP is reported to be downregulated in piglets at weaning, LPS from commensal and pathogenic gram-negative bacteria could increase inflammatory processes by TLR-4 activation, increasing diarrhea events during this phase. Although some studies had reported potential IAP roles to promote gut health, investigations about exogenous IAP effects or feed additives modulating IAP expression and activity yet are necessary. However, we discussed in this paper that the critical assessment reported can suggest that exogenous IAP or feed additives that could increase its expression could show beneficial effects to reduce diarrhea events during the post weaning phase. Therefore, the main goals of this review are to discuss IAP's role in intestinal inflammatory processes and present feed additives used as growth promoters that may modulate IAP expression and activity to promote gut health in piglets. (**Key Words:** Feed Additives, Intestinal Alkaline Phosphatase, Intestinal Inflammation, Gut Health, Swine)

INTRODUCTION

Alkaline phosphatases (APs) have been continuously and extensively investigated for over 50 years. The APs are a group of enzymes that hydrolyze phosphate monoesters at alkaline pH (Sussman et al., 1989). At least four AP isoforms have been described in mammalian cells: placental alkaline phosphatase, intestinal alkaline phosphatase (IAP), liver/bone/kidney (tissue-unspecific), and placental-like (expressed in the testis and thymus) (Goldstein et al., 1982).

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In piglets, the development of the gastrointestinal tract is a specific and dynamic process, since the intestinal epithelium is continuously exposed to microorganisms as well as variable feed types, such as sow milk and solid diet, containing different ingredients. After weaning, the diet changes from liquid to solid, with lower palatability and digestibility. This adaptation period is one of the reasons why the weaning phase is considered the most critical stage during swine production (Smith et al., 2010). Besides, early weaning promotes gut morphological and physiological changes, such as villi atrophy and crypt hyperplasia, and consequently, reduces the gut's ability to digest and absorb nutrients (Tucci et al., 2011). In this scenario, the post-weaning is associated with increased diarrhea incidence due the intestinal proliferation and mucosal attachment of the pathogenic *Escherichia coli*, combined with the immature immune of the piglet's intestine (Pié et al., 2004; Heo et al.,

2013).

Under homeostasis of the intestinal microbiota, IAP acts suppressing inflammatory responses from the host that may be induced by lipopolysaccharide (LPS) from commensal bacteria (Vaishnava and Hooper, 2007). However, the changes caused by weaning lead to reduced feed intake and gut morphological and physiological alterations resulting in lower expression level and activity of apical brush border IAP, thereby, decreasing the protective effects of IAP in the gut (Lackeyram et al., 2010; Lallès, 2010; Lallès, 2014).

Previous reports have suggested that IAP promotes several beneficial effects to the intestinal health of mammals, including prevention and reduction of intestinal inflammation and bacterial translocation, regulation of calcium absorption, and modulation of intestinal bacterial growth and local intestinal pH (Bates et al., 2007; Martínez-Moya et al., 2012; Alam et al., 2014; Brun et al., 2014; Malo et al., 2014). In this review, the mechanisms by which IAP contributes to gut health maintenance and prevention of bacterial infections are discussed. In addition, feed additives used as growth promoters that may modulate expression and activity of IAP to promote gut health in piglets are described.

OVERVIEW

The gastrointestinal tract is a complex segment formed by different structures, including the intestinal epithelium, which contains a variety of cells such as absorptive, Paneth, goblet, endocrine and microfold (M) cells. A healthy gastrointestinal tract permits the absorption of nutrients while maintaining the ability to respond appropriately to a diverse milieu of dietary and microbial antigenic components (Burkey et al., 2009). The host intestine is protected from the antigenic components by physical and chemical barriers formed by the gastrointestinal epithelium (Bevins et al., 1999). These barriers are reinforced by the innate and acquired mucosal immune response (McGhee et al., 1999), which play roles in maintaining a homeostatic balance between immune tolerance and responsiveness (Artis, 2008).

Recognition of pathogens is mediated by a set of germline-encoded receptors that are referred to as pattern-recognition receptors (PRRs). These receptors recognize pathogen-associated molecular patterns (PAMPs), which are shared by a large group of microorganisms, and initiate proinflammatory cytokines transcription. Toll-like receptors (TLRs) function as PRRs in mammals, and they play an essential role in the recognition of microbial components and innate immune response (Akira et al., 2001; Takeda and Akira, 2004; Vaishnava and Hooper, 2007). Currently, porcine TLR1-10 have been described in the literature (Shimosato et al., 2005;Tohno et al., 2005;Shinkai et al., 2006a,b; Tohno et al., 2006; Sang et al., 2008;Uenishi and Shinkai, 2009; Zhang et al., 2013). TLRs are largely divided into two subgroups depending on their cellular localization and specificity toward their respective PAMPs. One group is composed of TLR1, TLR2, TLR4, TLR5, TLR6, and TLR10, which are expressed on cell surfaces and recognize microbial membrane components such as lipids, lipoproteins, and proteins; the other group is composed of TLR3, TLR7, TLR8, and TLR9, which are expressed exclusively in intracellular vesicles, and they recognize microbial nucleic acids (Cario, 2005). In general, after expressed the TLRs dimerize and undergo conformational changes to bind to their respective PAMPs through interaction of toll/interleukin 1 (IL-1) receptor domain-containing adaptor molecules such as the myeloid differentiation primary response gene 88 (MyD88) (Yamamoto et al., 2002; Oshiumi et al., 2003). The interaction between TLRs and MyD88 characterize the pathway MyD88-dependent that seems to be essential to all TLRs. However, peculiarly TLR3 and TLR4 signaling can follow a pathway MyD88-independent (Takeda and Akira, 2004). While there are several mechanisms by which these changes may occur, the common final response to these events is the release of cytokines, chemokines, and the recruitment of inflammatory cells. In this regard, nuclear factor κ-light-chain-enhancer of activated B cells (NF-κB) and interferon regulator factor (IRF)-3 play a pivotal role in intestinal inflammation by controlling the expression of proinflammatory mediators and stimulating the secretion of epithelial fluid, electrolytes, immune receptors, and cell surface adhesion molecules (Berkes et al., 2003; Takeda and Akira, 2004).

During an inflammatory response, epithelial cells can act increasing *IAP* expression and to modify proinflammatory mediators induced by microbial molecules. For example, the brush border enzyme IAP can dephosphorylate LPS and reduce their toxicity (Koyama et al., 2002; Beumer et al., 2003; Van Veen et al., 2005; Tuin et al., 2006). Besides, the IAP localized on apical surface of the villi can potentiate their effects. In addition, bacterial toxins can also upregulate the expression of host-derived products such as galanin, nitric oxide, and prostaglandins, which increase chloride secretion in the epithelial cells and promote diarrhea (Berkes et al., 2003). However, exogenous IAP could promote growth of intestinal commensal bacteria (Malo et al., 2014), re-establishing the intestinal microbiota homeostasis and consequently increasing bacterial competition. Thus, IAP can have direct (against LPS) and indirect (increasing commensal bacterial growth) effects to control intestinal disorders.

Bacterial translocation to organs such as liver, spleen, and mesenteric lymph nodes occurs in consequence to intestinal inflammatory processes, most likely because of

alterations in gut permeability (Martínez-Moya et al., 2012; Alam et al., 2014). This suggests that enteric bacterial infections cause increased intestinal permeability, fluid and electrolyte secretion. The mechanism by which IAP prevents inflammatory processes and maintains homeostasis to promote intestinal health in the host will be discussed in the following sections.

IAP ACTIVITY INHIBITS THE GASTROINTESTINAL INFLAMMATORY CASCADE AND SUPPRESSES BACTERIAL TRANSLOCATION

TLR4 recognizes LPS present in the outer membrane of gram-negative bacteria and induces host immune response and injury repair (Abasht et al., 2008), resulting in recruitment of macrophages (Beutler and Rietschel, 2003), activation of NF-κB and IRF-3 (through MyD88-dependent and independent pathways) (Takeda and Akira, 2004; Gao et al., 2014) and consequently, releasing inflammatory mediators such as tumor necrosis factor α (TNF-α) and ILs. These cascades induce localized neutrophil action against the infection (Beumer et al., 2003; Bates et al., 2007; Mussá et al., 2013).

IAP can dephosphorylate LPS on the outer membrane of gram-negative bacteria, thereby reducing its toxicity (Poelstra et al., 1997a,b; Koyama et al., 2002; Bates et al., 2007; Chen et al., 2011). This mechanism is used as a host response to LPS, where animals submitted to a LPS challenge show upregulation of *TLR4* and *IAP* gene expression (Koyama et al., 2002; Bates et al., 2007; Abasht et al., 2008; Goldberg et al., 2008). For instance, reduced TNF-α release was noted in piglets treated with LPS plus IAP, compared to piglets treated with LPS alone (Beumer et al., 2003), suggesting that the enzymatic action of IAP suppressed the LPS-induced inflammatory processes. These studies confirm the role of IAP in detoxifying LPS in the intestinal lumen by removing its phosphate residues.

Unmethylated cytosine-guanosine dinucleotides (CpG; a component of bacterial DNA) and flagellin (a protein found in both gram-negative and gram-positive bacterial flagella) can also induce host inflammatory responses and may be IAP targets (Chen et al., 2010). These compounds are recognized by the host through TLR9 and TLR5, respectively (Shinkai et al., 2006a; Tohno et al., 2006). Dephosphorylation of bacterial CpG DNA and flagellin by IAP inhibits the induction of IL-8 proinflammatory cytokines, mediated by TLR recognition (Chen et al., 2010). Furthermore, IAP dephosphorylates uridine diphosphate (UDP), a nucleotide that upregulates the expression of *IL-8*, which is released into the gut by the host under inflammatory conditions (Moss et al., 2013).

Administration of IAP via the oral route to mice with induced-colitis normalized the expression levels of inflammatory markers and resulted in lower rates of bacterial translocation across the gut epithelium, compared to control and antibiotic-treated (Martínez-Moya et al., 2012). Therefore, there is viability to oral route administration, which indicates the potential therapeutic effects of IAP in suppressing the inflammatory cascade and reducing the risk of sepsis (Bentala et al., 2002).

The stress imposed on piglets during weaning and their immature digestive physiology makes weaning the most critical phase in swine production. Decreased performance may be attributed to the reduced passive immunity in piglets and the adaptive period to the solid diet (Pié et al., 2004). There are reports of increased incidence of opportunist enteric bacterial infections and diarrhea during this stage (Fairbrother et al., 2005; Taras et al., 2006). Weaning and the associated feed intake reduction are known to cause lower intestinal villi height and increased crypt depth, compromising the intestinal integrity (Goldberg et al., 2008; Lallès et al., 2010). Downregulation of the *IAP* gene and the consequent absence of the protective effect of IAP are associated with poor intestinal integrity in weaned piglets (Lackeyram et al., 2010). Therefore, a better understanding of the factors that regulate the expression of *IAP* and promote gut health by reducing inflammatory processes and maintaining gut integrity may aid in the prevention and treatment of enteric diseases in piglets.

IAP REDUCES THE INDUCTION OF FLUID AND ELECTROLYTE SECRETION IN INTESTINAL INFLAMMATORY

A healthy intestinal epithelium has the capacity to control fluid and electrolyte secretion. Regular transport of Cl^-, Na^+, and K^+ helps to maintain the intra- and extra-cellular osmotic balance. Chloride secretion promotes paracellular sodium movement and potassium influx, while sodium luminal accumulation promotes osmotic water diffusion. Under bacterial infections and inflammatory conditions, this complex regulation mechanism is disrupted and inflammatory diarrhea can occur (Berkes et al., 2003). Hemodynamic responses resulting in dehydration, hypotension, poor tissue perfusion, and multi-organ failure are possible consequences of this disruption (Howe, 2000; Uysal et al., 2000).

Besides LPS, heat stable bacterial enterotoxins can also mediate cyclic adenosine monophosphate- and cyclic guanosine monophosphate-dependent cystic fibrosis transmembrane conductance regulator and calcium/chloride channels, thereby modifying the osmotic gradient and increasing water release from cells into the intestinal lumen

(Eisenhut, 2006). According to Malo et al. (2014), IAP activity positively modulates the growth of commensal bacteria by inducing adenosine triphosphate (ATP) dephosphorylation, leading to increased competition with potential pathogens. Therefore, this increased competitiveness by commensal bacteria may directly reduce the production of enterotoxins by pathogenic/opportunistic bacteria.

FEED ADDITIVES USED AS GROWTH PROMOTERS THAT INCREASE IAP GENE EXPRESSION AND ACTIVITY

Early weaning is common practice in swine production in order to enhance sow productivity. To reduce intestinal damage and diarrhea in piglets during the weaning period, feed additives are included in their diet. The effects of various feed additives have been studied extensively after the prohibition on use of antibiotics as growth promoters by the European Union in 2006, due the possible antibiotic-induced bacterial resistance and cross-contamination to humans (Gallois and Oswald, 2008; de Lange et al., 2010; Heo et al., 2013).

Sodium butyrate (NaBu), essential oils, and zinc are some of the feed additives that induce IAP expression and/or activity (Malo et al., 2006; Prakash and Srinivasan, 2010; Martin et al., 2013). Moreover, these additives modulate the gastrointestinal microbiota of pigs (Bederska-Łojewska and Pieszka, 2011).

Perez et al. (1998) reported that NaBu modulates the immune system by increasing TNF-α and COX2 production. Weber and Kerr (2008) demonstrated *in vivo* that NaBu modulated the immune response in mesenteric lymph nodes of LPS-challenged pigs by upregulating the expression of *IL-6*. Malo et al. (2006) suggest that NaBu upregulates *IAP* expression. According to Bol-Schoenmakers et al. (2010), soluble IAP did not reduce NF-κB activation by LPS-induction in epithelial cells, but NaBu inducing *IAP* expression reduced inflammatory response to LPS. The authors concluded that endogenous IAP can be sufficient to ameliorate moderate inflammation induced by LPS, but exogenous IAP is necessary to have a beneficial effect towards severe intestinal damage. Although pre-existing proinflammatory cytokines may inhibit endogenous *IAP* expression, NaBu could reverse it through an unclear mechanism, suggesting the potential preventive application of NaBu.

Another alternative to antibiotic growth promoters is zinc. When zinc is used in therapeutics doses (as zinc oxide), it can improve animal performance (Shelton et al., 2011; Hu et al., 2013; Martin et al., 2013). Zinc supplementation upregulates the expression of genes involved in growth and differentiation of intestinal mucosal cells (transforming growth factor β1, insulin-like growth factor 1 [IGF-1], and IGF-1 receptors), reduces bacterial adhesion and translocation, prevents changes in intestinal barrier function, modulates the immune system by reducing the levels of inflammatory cytokines (IL-8 and interferon γ), upregulates the expression of anti-inflammatory cytokines (IL-10), reduces luminal ATP concentration, modulates intestinal pH, and gut microbiota (Roselli et al., 2003; Li et al., 2006; Hu et al., 2013; Martin et al., 2013). Recently, Kim et al. (2012) and Martinet al. (2013) reported the effect of zinc increasing *IAP* gene expression, which suggest that the zinc-inducing IAP overexpression may contribute to enhanced intestinal health.

Essential oils are another class of additives that modulate IAP activity and gene expression. Dietary black pepper, piperine, red pepper, capsaicin, and ginger are also known to increase IAP activity (Prakash and Srinivasan, 2010). In contrast, sage oil extract decreased IAP activity in chicken jejunum (Levkut et al., 2010). Similar results were observed in the case of oregano essential oil (Levkut et al., 2011). However, a commercial blend of essential oil containing thymol did not modulate IAP activity in broiler proximal intestine (Jang et al., 2007), probably because of the strong antimicrobial activity of the blend essential oils. Although essential oils had been reported modulating IAP activity and expression, their effects need be investigated in studies using swine as experimental model.

CONCLUSION

There is growing scrutiny on animal production to minimize the use of antibiotics. Several studies have revealed that IAP might be a potential gut health promoter. There is also evidence that some feed additives, highlighted in this review, can modulate IAP expression and/or activity. Although, IAP expression and/or activity are altered as result of the interplay among dietary factors, microbiota, and the host status, very few studies have investigated IAP effects and its modulation by feed additives in swine, especially to reduce diarrhea events during post weaning. However, available experimental data support the role of IAP in catalyzing the breakdown of monophosphates and detoxifying bacterial LPS, CpG DNA, and flagellin as well as extracellular nucleotides, such as UDP, resulting in lower TLRs activation and regulating inflammation and gut microbiota.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial organization regarding the material discussed in

the manuscript.

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