

A summary of feed additives, intestinal health and intestinal alkaline phosphatase in piglet nutrition

JANSLER LUIZ GENOVA^{1*}, ANTONIO DIEGO BRANDAO MELO², PAULO EVARISTO RUPOLO¹, SILVANA TEIXEIRA CARVALHO¹, LEANDRO BATISTA COSTA², PAULO LEVI DE OLIVEIRA CARVALHO¹

¹Animal Science Department, State University of Western Paraná, Marechal Cândido Rondon, Brazil

²Animal Science Department, Pontifical Catholic University of Paraná, Curitiba, Brazil

*Corresponding author: jansllerg@gmail.com

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Abstract: Weaning is considered the “critical window” in the piglet’s life because it is associated with several stress factors, such as loss of contact with the mother and original litter, solid diet, environmental and structural changes, and the establishment of a new hierarchy. During this abrupt period, several events such as reduced feed intake, high morbidity, susceptibility to enteric infections and post-weaning diarrhoea are observed. The nutritional landscape of the piglet gut is modified, which can compromise the maturity of the gastrointestinal system, the stable intestinal microbiome and the active immunity developed as an indicator of intestinal health. However, with increased awareness of feed safety issues and the development of drug-resistant bacteria, the interest in producing pigs without the use of antimicrobial growth promoters (AGP) is increasing, since long-term use and therapeutic doses of AGP can contribute to the reduction of bacterial diversity and increase of inflammatory bowel disease (IBD). Thus, the most widely researched alternatives include the use of feed additives, feeding strategies, nutraceuticals/functional foods and available handling that can reduce the risk of IBD beyond basic nutritional functions. Studies have reported intestinal alkaline phosphatase as a new nutritional therapy associated with intestinal health which may be a “key additive” in the AGP replacement. In this review article, the purpose is to show some current aspects of feed additive research, addressing a concept of the “intestinal health” from different points of view and properties of alkaline phosphatase.

Keywords: alkaline phosphatase; gastrointestinal health; intestinal microbiota; immune system; weanling piglet

Introduction

Recently, the “gastrointestinal tract health” has been greatly investigated despite the lack of a clear definition of the term or its aetiology, although in general terms, the “intestinal health” encompasses a series of physiological and functional characteristics (Pluske et al. 2018). Thus, in recent decades, a great deal of research has been devoted to the development of alternatives to antimicrobial growth promoters (AGP) to maintain the health

and growth performance of pig. The most widely researched alternatives include organic and inorganic acids, high levels of zinc oxide, essential oils, herbs and spices, some types of prebiotics, probiotics, bacteriophages, antimicrobial peptides, feeding strategies and available handling that influence or intend to influence different aspects of intestinal health (Adewole et al. 2016; Jayaraman and Nyachoti 2017; Liu et al. 2018).

Current studies have reported the potential therapeutic effect of the alkaline phosphatase enzyme

in decreasing the inflammatory bowel disease (IBD) (Bilski et al. 2017) and metabolic diseases such as insulin resistance (Gul et al. 2017), in reducing vascular calcification and improving cardiovascular outcomes in patients with chronic kidney disease or type 2 diabetes mellitus (Haarhaus et al. 2017), use in the diagnosis of bone tumours such as osteosarcoma (Agustina et al. 2018; Gu and Sun 2018), as a prognostic marker for patients with prostate cancer (Heinrich et al. 2018) and upper tract urothelial carcinoma (Tan et al. 2018).

Besides that, previous studies have demonstrated the effect of intestinal alkaline phosphatase (IAP) isoform on improving the intestinal health of animals (Lalles 2014; Melo et al. 2016; Bilski et al. 2017; Rader 2017) through the dephosphorylation of bacterial lipopolysaccharide (LPS), with reduction of inflammatory processes in the gut (Beumer et al. 2003; Bates et al. 2007), dephosphorylation of luminal ATP, acting as a prebiotic in regulating the growth of commensal bacteria (Alam et al. 2014; Malo et al. 2014), modulation of intestinal pH (Akiba et al. 2007), inorganic phosphate homeostasis (Sasaki et al. 2018), dephosphorylation of nucleotides involved in inflammatory processes

(Moss et al. 2013), in addition to reducing bacterial translocation (Martinez-Moya et al. 2012).

Thus, IAP can be an alternative to AGP with potential effect in the post-weaning period of piglets. Therefore, the aim of this review is to describe current findings on the use of feed additives, the main approaches to intestinal health and the therapeutic effect of IAP as an alternative to AGP in pig nutrition.

The use of feed additives in piglet nutrition with the role of healthy gastrointestinal tract

Recent studies use a range of products and handling alternatives such as feed additives (Table 1) and feeding strategies (Adewole et al. 2016; Liao and Nyachoti 2017; Zhai et al. 2018) in order to improve the growth performance, minimizing the use of AGP and inaccessible feed ingredients.

The wide number of feed additives for use as alternatives or substitutes to AGP that have been evaluated in pig nutrition generally aims to improve immune responses and effective immune

Table 1. Current results with the addition of feed additives in piglet diets

Items	Body weight (kg)	Days of age	Additive dose	Main effects	References
Enzyme (<i>Buttiauxella</i> phytase)	11.0 ± 1.5	21 and 28	500, 1 000 or 2 000 FTU/kg diet	increased ADG and G:F, linear response on nutrient digestibility and reduced P and Ca ²⁺ excretion	Dersjant-Li et al. (2017)
Enzyme (carbohydrase blend: cellulase, β-glucanase, and xylanase)	6.43 ± 0.06	–	0.01%	enhanced growth rate, improved small intestinal barrier integrity and reduced immune activation	Li et al. (2018)
Enzyme (xylanase)	6.43 ± 0.06	–	0.01%	reduced ATTD of NDF and ADF	Li et al. (2018)
Enzyme (phytase)	5.6 ± 0.5	–	500 or 2 000 FTU/kg diet	2 000 FTU/kg: increased ADG, ADFI, feed efficiency, plasma inositol concentration and expression of GLUT4	Lu et al. (2019)
Essential oil (thymol 50% and carvacrol 50%)	6.5 ± 0.9	–	100 mg/kg diet	decreased the intestinal oxidative stress and influenced microbial populations	Wei et al. (2017)
Essential oil (thymol 25% and carvacrol 25%)	8.64 ± 0.33	–	30 mg/kg diet	improved nutrients digestibility, intestinal morphology, digestive enzymes and ↑ Lactobacilli counts of faeces	Xu et al. (2018)
Organic acid (sodium butyrate salt)	8.3 ± 0.32	24	2.1 g/kg diet	reduced colonization and shedding of <i>Salmonella</i>	Barba-Vidal et al. (2017a)
Organic acid (SCFA: formic, acetic and propionic acid combined with MCFA)	8.63 ± 1.56	–	2 000 or 3 000 mg/kg diet	reduced the incidence of diarrhea and faecal <i>E. coli</i> counts, ↑ nutrients digestibility and positive effects on serum immunity	Long et al. (2018)

Table 1 to be continued

Items	Body weight (kg)	Days of age	Additive dose	Main effects	References
Organic acid (MCFA and AO)	8.09 ± 0.11	28	0.2%, 0.4% or 0.6%	combinations of organic acids could improve growth performance, reduce post-weaning diarrhea, and enhance serum immunity	Han et al. (2018)
Organic acid (fumaric acid 17%, citric acid 13%, malic acid 10% and 1.2% MCFA)	6.54 ± 0.78	–	0.1% or 0.2%	improved growth performance and nutrient digestibility	Upadhaya et al. (2018)
Organic acid (benzoic acid 50%, calcium formate 3%, fumaric acid 1%)	8.64 ± 0.33	–	1.5 g/kg diet	improved nutrients digestibility, faecal score, intestinal morphology, ↑ Lactobacilli counts of faeces and butyric and valeric acid concentration	Xu et al. (2018)
Plant extract (<i>Eucommia ulmoides</i>)	7.22 ± 0.34	21 ± 2	0.5% or 6%	improved growth performance, jejunal morphology and changed colonic microbial composition	Peng et al. (2019)
Prebiotic (galacto-oligosaccharides)	1.55 ± 0.05	–	10 ml (1 g GOS/kg BW)	increased ADG, decreased crypt depth, increased the jejunal lactase, maltase and sucrase activity, facilitated the mRNA expression of SGLT1 and GLUT2	Tian et al. (2018)
Prebiotic (β-glucans, glucomannans and MOS)	6.32 ± 0.10	21	1 000, 2 000, or 3 000 mg/kg diet	3 000 mg/kg reduced the jejunal villus density increased spleen weight	Anjos et al. (2019)
Prebiotic (β-glucan)	7.3 ± 0.2	–	0.25 g/kg diet	did not improve gut health	Mukhopadhyaya et al. (2019)
Probiotic (<i>Bacillus licheniformis</i>)	8.3 ± 0.32	24	10 ⁹ CFU/kg diet	reduced colonization and shedding of <i>Salmonella</i> , effect on behavioral displays	Barba-Vidal et al. (2017a)
Probiotic (<i>Bifidobacterium longum</i> subsp. <i>infantis</i> CECT 7210) [†]	7.9 ± 0.05 and 6.8 ± 0.19	24 ± 4 and 21 ± 2	10 ⁹ CFU which was supplemented in a 2 ml solution	reduced the faecal excretion of <i>Salmonella Typhimurium</i> and the mucosal colonization of coliforms in the ETEC K88 trial, produced a stimulation of the intestinal immune system	Barba-Vidal et al. (2017b)
Probiotic (<i>Bacillus licheniformis</i> and <i>Saccharomyces cerevisiae</i>)	4.9 ± 0.4	21 ± 3	500 mg/kg diet	alleviated the ↑ in the endotoxin and diamine oxidase concentration, and caecal <i>E. coli</i> count	Pan et al. (2017)
Probiotic (<i>Bacillus amyloliquefaciens</i>)	14.57 ± 0.25	–	2 × 10 ⁸ CFU/kg diet	increased the activities of amylase, disaccharides and Na ⁺ /K ⁺ -ATPase, maintained the intestinal integrity and decreased activity of diamine oxidase	Hu et al. (2018)
Probiotic (<i>Clostridium butyricum</i>)	–	28	2 × 10 ⁶ or 5 × 10 ⁵ CFU/g diet	did not significantly reduce faecal excretion, serological response and intestinal carriage	Peeters et al. (2019)

ADF = acid detergent fibre; ADFI = average daily feed intake; ADG = average daily gain; AO = acid organic; ATTD = apparent total tract digestibility; BW = body weight; CFU = colony-forming units; ETEC = enterotoxigenic *Escherichia coli*; FTU = phytase units; G : F = gain : feed ratio; GLUT2 = glucose transporter type 2; GLUT4 = glucose transporter type 4; MCFA = medium-chain fatty acids; mRNA = messenger RNA; NDF = neutral detergent fibre; SCFA = short-chain fatty acids; SGLT1 = sodium glucose co-transporter 1

[†]two experiments

status, reducing a pathogenic microorganism load in the gastrointestinal tract, stimulate the establishment of mutualistic or commensal microorganisms in the gastrointestinal tract (GIT) and/or stimulate a digestive process (De Lange et al. 2010).

According to Adewole et al. (2016), feed additives have been used by several researchers and in research models for the purpose of improving growth performance and also to prevent diseases, but the effectiveness of these additives depends mainly on the amount added to the diet. Along the same line of reasoning, Celi et al. (2017) reiterated that the reason for this inconsistency may be that the effectiveness of each additive depends on diet (e.g. composition, diet processing and feeding methods), colonization and associated succession of microbial populations, stress and genetics. Therefore, it is not possible to recommend a specific additive that has positive effects on all diets, but it is likely that if no AGP is used, at least some additives are beneficial in diets fed to piglets (Liu et al. 2018).

Pluske et al. (2018) reported that additives are predominantly characterized not only by their different modes of action, but also by the variation in responses obtained when added to pig diets. This variation is presumably a consequence, in part, of many different management conditions pigs are subjected to, which in turn influence factors such as microbiota composition and intestinal mucosal immunity.

Regarding the use of essential oils (EOs) in pig nutrition, Stevanovic et al. (2018) argued that the chemical composition of EOs depends on plant genetics, plant growth conditions, harvest developmental stage and extraction processes of active compounds. Moreover, their biological effects are influenced by the interaction of phytochemicals and their bioavailability in the animals' GIT. In addition, research with EOs should focus on reliable methods to identify and control the quality and effects of EOs.

Additional studies should be conducted on phyto-genic additives (PAs) or simply phytobiotics in order to verify their effects on the intestinal health of piglets, as a systemic approach is required to explain the role of these PAs in terms of the type and dose of each additive. Besides that, the potential benefits of phytobiotics may differ due to the wide variation in the plant diversity, composition and active principles, which results in diffi-

culty in comparing the efficiency of different PAs (Suryanarayana and Durga 2018).

Regarding the prebiotics, Liu et al. (2018) further highlighted a possible positive impact on the immune and microbiological system, but additional research is needed to document these effects. It is also possible that the use of prebiotics, direct-fed microorganisms, yeast and nucleotides may have positive impacts on the growth performance of pigs, but the results have been inconsistent, as the efficiency of each additive depends on diet and also on the health status of the animals (Liu et al. 2018).

The aforementioned authors also reported that, despite many years of research, the exact mode of action of dietary acidifiers was not fully elucidated yet and further investigations were needed. However, according to Kil et al. (2011) and Pearlin et al. (2020), the most frequently reported mechanisms include GIT pH reduction, and thus they can affect the growth of pathogenic bacteria, besides having a role in improving the nutrient digestion and gut health.

Corroborating previous reports, the addition of exogenous enzymes to piglet diets has focused on understanding and determining the effects on growth performance parameters and nutrient digestibility. However, there is a need to clarify the effects of feed enzymes on aspects involving the intestinal health (Bedford and Cowieson 2012). In this sense, several mechanisms how enzymes act on the intestinal microbiota have been clarified (Kiarie et al. 2013), but the extent to what these effects may contribute to the overall health of GIT is still unknown.

Thus, there is a need for additional research in order to validate the findings. An important point to be discussed in this review is the role of feed enzymes as possible alternatives to AGP, as exogenous enzymes can improve intestinal stability by reducing substrates for putrefying organisms, by increasing substrates for beneficial fermentative organisms and the ability of the gut to defend itself against unwanted bacterial ingress (Celi et al. 2017).

In this aspect, the potential use of enzymes in order to promote an improvement in the intestinal health of piglets and to reduce diarrheal events during the post-weaning phase requires studies and information. Melo et al. (2016) reported that, although some studies verified the potential roles of IAP in the intestinal health like a marked reduction in the occurrence of diarrhoea and modulation

of intestinal indicators (data not yet published), investigations on the exogenous effects of IAP or feed additives modulating IAP expression and activity are still needed. In summary, inconsistent results obtained with feed additives may be due to differences in the pig age, health status, environmental conditions or available handling. Therefore, the research on alternatives to AGP and the development of new efficient and safe alternatives will be a long process (Cheng et al. 2014).

Physiological mechanisms and the health of the gastrointestinal tract

The health of the GIT (“intestinal health”) is a commonly used term and currently it is a matter of enormous interest in research on piglets (Pluske

et al. 2018). “Intestinal health” is a reflection of several interactions between the animal and its environment (Figure 1).

According to Kogut and Arsenault (2016), GIT health is defined as the absence and/or prevention of diseases for the animal to be able to perform its physiological functions to resist exogenous and endogenous stressors. Corroborating this idea, Bischoff (2011) defined five main criteria that could form the basis of a comprehensive definition of intestinal health, which would be: effective digestion and absorption of dietary nutrients; absence of IBD; optimal and stable intestinal microbiota/microbiome; effective and active immune status, and indicator of animal welfare.

In this sense, the abrupt post-weaning period in piglets not only causes structural and functional changes accentuated in the small bowel, but also

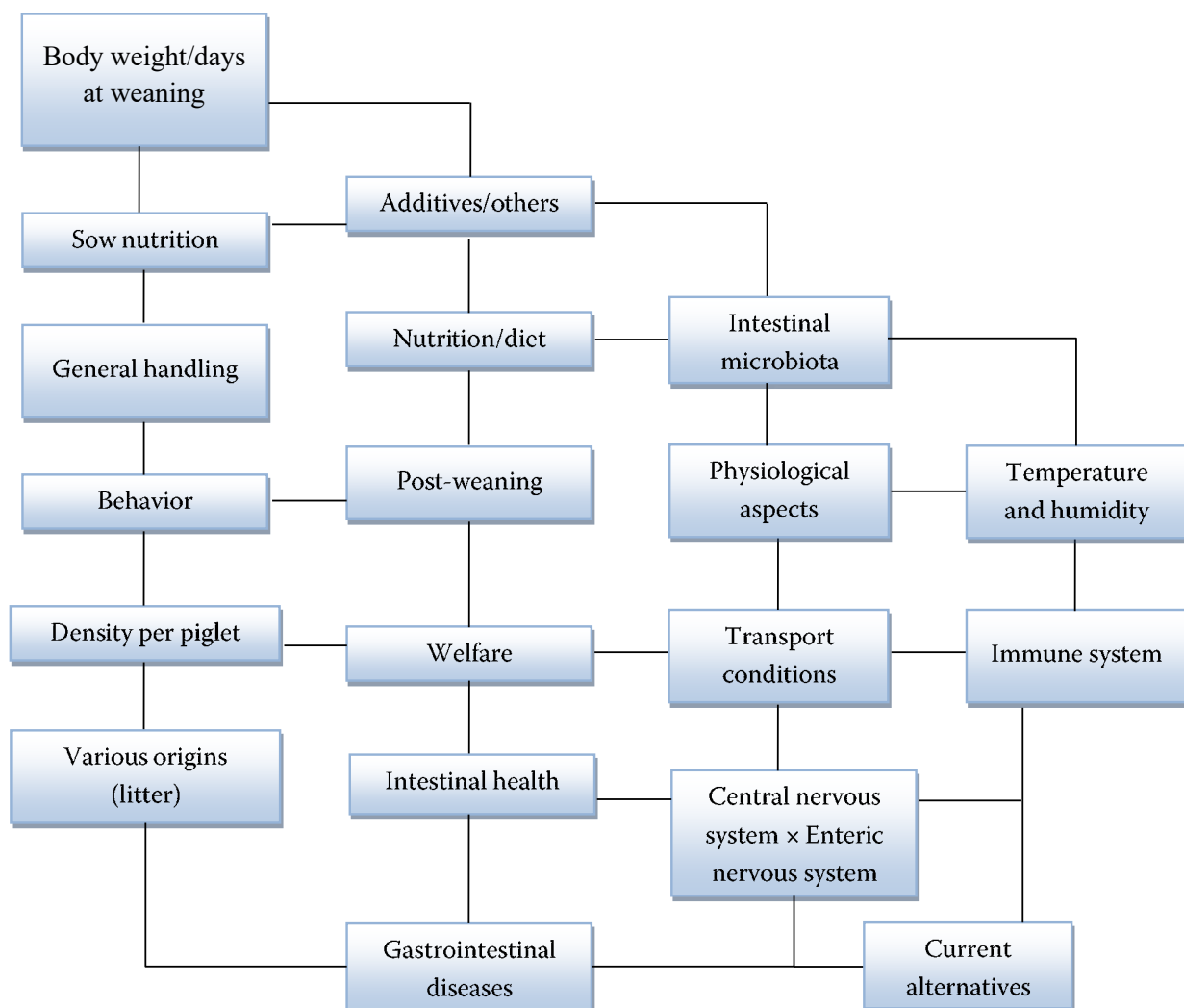


Figure 1. Fundamental components for promoting the intestinal health of piglets during the nursery phase

contributes to an inflammatory bowel process that, in turn, compromises the villus and crypt architecture (Pluske et al. 2018). Jayaraman and Nyachoti (2017) argued that inadequate management practices may result in reduced feed intake, stress and predisposition to disease conditions, consequently affecting intestinal health and performance of weaned piglets.

However, the functions of the GIT extend beyond the processes associated with feed intake or effective diet, nutritional particle digestion and concomitantly active or passive absorption and intestinal barrier function. GIT plays an important role in the regulation of epithelial, physiological and immunological functions, and host interaction with the resident microbiota (Celi et al. 2017).

When the intestinal health approach is related to nutritional management, it is important to keep in mind that associated with nutrition it can modulate the immune function in the GIT through several distinct mechanisms such as cytokine production and regulation of the gut barrier function (Celi et al. 2017). Furthermore, it may influence the composition and metabolic activity of the GIT microbiota as a consequence of changes in substrates available for microbial fermentation (Yeoman and White 2014).

Studies conducted with other species showed that dietary components may alter intestinal permeability (David et al. 2014; Kelly et al. 2015). In particular, high-fat diets are associated with a greater lipopolysaccharide (LPS) translocation through the intestinal wall (Moreira et al. 2012). In addition, meals rich in fibre and fruit have been shown to reduce meal-induced increases with high fat/carbohydrate content at LPS plasma levels, inflammatory response and Toll-like receptor (TLR) 2 and 4 expression (Ghanim et al. 2009).

In this context, the effects of diet on the gastrointestinal health can be directed to different functions of GIT. At the same time, the effects of the central nervous system on the microbiota composition are probably mediated by a disturbance of the normal luminal/mucosal habitat that can also be restored by diet (Carabotti et al. 2015). Ultimately, “intestinal health” represents the outcome of GIT in response to its ability to react and adapt to the insults and challenge models it encounters (Adewole et al. 2016; Pluske et al. 2018).

Moreover, the gut microbiota (or microbiome, representing the genomic information of the mi-

crobial, intestinal ecology) represents a compromise between useful barrier functionality, synthesis of beneficial nutrients and proteins, better energy acquisition, action on the deleterious effects of inflammation and subclinical/clinical pathologies (Celi et al. 2017), influence on the functional diversity of B and T cells, with emphasis on differentiation of IgA-producing B and T cells that carry the CD4 antigen (Honda and Littman 2016).

Other research has reported that the association between the enteric nervous system (ENS) and the upper centres via the parasympathetic nervous system and/or the endocrine system also plays a fundamental role in the animal welfare, intestinal health and the structure/integrity and function of the GIT (Moeser et al. 2017), i.e., the complex interactions that occur in the GIT between nutrition, mucosa (epithelium) of the GIT and intestinal microbiota are essential to affect intestinal health (Pluske et al. 2018), although the precise mechanisms how the GIT microbiome contributes to changes in behaviour and central nervous system effects are less evident (Foster et al. 2017).

In general, GIT health is a complex system that includes several factors, but it is of paramount importance to highlight gastrointestinal barriers, in which it is formed by a multilayered system of host defence mechanisms (e.g. GI epithelium barrier, GI immune system barrier and ENS barrier), GIT microbiota/microbiome and nutritional management (Adewole et al. 2016; Celi et al. 2017; Jayaraman and Nyachoti 2017; Moeser et al. 2017; Barba-Vidal et al. 2018; Liu et al. 2018; Pluske et al. 2018).

The intestinal barrier is mainly formed by a layer of epithelial cells attached by tight-junction proteins called tight junctions, consisting mainly of transmembrane protein complexes (i.e. claudins and occludins) and cytosolic proteins ZO (i.e. junctional adhesion molecule, ZO-1, ZO-2 and ZO-3) (Liu et al. 2018). ZO-1 and occludin are key tight-junction proteins, and levels of these proteins are consistently associated with the intestinal barrier function (Song et al. 2015).

As described by Pluske et al. (2018), the intestinal barrier function along with the mucosal immune system are continually challenged by external (i.e. diet provided) and internal (i.e. intestinal microbiota) factors, as well as several cell types such as dendritic cells, lymphocytes (adaptive or acquired immune system), macrophages and cytokines (innate or non-specific immune system) have evolved to perform

important roles in regulating the communication between the GIT microbiome and its mucosal immune system.

Moeser et al. (2017) reported that the intestinal epithelial barrier is also supported by other types of specialized epithelial cell, such as goblet cells which provide a protective mucous layer and Paneth cells that secrete antimicrobial peptides. In addition, enteroendocrine cells play important roles in the pathogen detection and can synthesize and release neuropeptides (i.e. serotonin and peptide YY, PYY), which have a diverse range of physiological functions from pathogen defence to metabolic regulation of appetite (Duca et al. 2013; Moeser et al. 2017). In short, intestinal epithelial cells act as immune sentinel cells, recognizing pathogenic signalling molecules and secreting interleukins (IL) and growth factors (i.e. IL-17A, IL-33, IL-23 and transforming growth factor- β) which have important immunomodulatory properties (Schiering et al. 2014; Moeser et al. 2017; Pluske et al. 2018).

Another mechanism that acts directly to promote this intestinal health is the ENS barrier, through the constant release of a series of neurochemicals it plays a central role in the motility, secretion and absorption of the gut and the modulation of epithelial permeability. Furthermore, the nervous system is also one of the main regulators of local gastrointestinal systemic and immune responses via neuroimmune synapses and can modulate the sensitivity and adherence of bacterial toxins (Moeser et al. 2017). There is a growing interest in studying the neuronal-immune communication as a way to explain mechanisms of gastrointestinal diseases.

From this point of view, it is evident that numerous factors influence the diversity and activity of the GIT microbiota, including colonization and associated succession of microbial populations (Pluske et al. 2018), as well as the interaction between the microbiota and the gut-brain axis (GBA) through the signalling from gut microbiota to brain, as well as in the opposite direction by means of neural, endocrine, immune and humoral connections (Carabotti et al. 2015).

Finally, studies are needed to clarify the role of the GIT microbiota in the relationships between animal nutrition (diet), physiology (digestion and absorption), health (immunology) and welfare (GBA). Celi et al. (2017) mentioned that the multifaceted and widely unknown interactions between microbial

populations, and the GIT microbiota and host, add another complexity level to this research area.

Positive impacts of alkaline phosphatase on piglet intestinal health

In current scientific literature, there are few studies conducted with the use of IAP in piglet diets, as well as the knowledge of its importance in the intestinal health of the host. In this introductory part, we discuss in general the main problems that the alkaline phosphatase enzyme can alleviate and report each of them.

However, alkaline phosphatases (ALPs) are a group of isoenzymes present in different body tissues and with distinct physicochemical properties. According to Lowe and John (2018), ALPs are located on the outer layer of the cell membrane in order to catalyze the hydrolysis of organic phosphate esters present in the extracellular space. In the liver, ALP is cytosolic and present in the hepatocyte canalicular membrane. ALP is present in decreasing concentrations in the placenta, ileal mucosa, kidneys, bones and liver, but the majority of IAP in the serum (more than 80%) is released from the liver and bone and in small amounts from the bowel, presenting high expression in the intestinal villi (Sussman et al. 1989). In recent reviews, Fawley and Gourlay (2016) and Haarhaus et al. (2017) reported that there are four ALP isoenzymes (Table 2).

LPS is characterized as the most abundant glycolipid complex present in the outer membrane of Gram-negative bacteria (i.e. *Escherichia coli*). It is a complex molecule, negatively charged, composed of a polysaccharide chain called the O-specific chain and a lipid portion called lipid A. Lipid A, present in the LPS molecule, expresses its endotoxic activity, and this fraction is responsible for stimulating the innate immune response of the host (Akira et al. 2001; Beumer et al. 2003).

The pathogen recognition by the host immune system is mediated by pattern recognition receptors (PRR), which refer to molecular patterns associated with a wide group of pathogenic microorganisms. TLR function as PRR and play an important role in the host immune system (i.e. development of antigen-specific adaptive immunity), such as pathogen recognition, signalling and inflammatory response induction (Werling and Jungi 2003; Takeda et al.

Table 2. Description of alkaline phosphatase (ALP) isoenzymes and isoforms

Gene	Protein	Isoforms	Localization	Functions
<i>ALPL</i>	ALP, tissue-nonspecific isozyme (TNALP)	bone-specific TNALP (B/l, B1, B1x and B2)	skeletal tissue, kidney, neutrophil granulocytes, developing nervous system, and other cell types (vascular cells)	(1) involved in skeletal mineralization, (2) vitamin B6 metabolism and (3) hypophosphatemia (genetic absence)
		liver-specific TNALP (L1, L2 and L3)	liver	unknown so far
<i>ALPI</i>	ALP, intestinal-type (IAP)	unknown so far	mainly in duodenum	(1) involved in fat absorption, (2) detoxification of LPS and free nucleotides, and (3) regulation of intestinal microbiota
<i>ALPP</i>	ALP, placental-type (PALP)	unknown so far	syncytiotrophoblasts, several tumours	(1) tumor marker and (2) detoxification of bacterial toxins
<i>ALPPL2</i>	ALP, placental-like (PLALP)	unknown so far	testis, malignant trophoblasts, testicular cancer	unknown so far

Source: Adapted from Fawley and Gourlay (2016); Haarhaus et al. (2017)

2003; Takeda and Akira 2004). According to Takeda et al. (2003), the inflammatory response induced by TLRs is dependent on a common signalling pathway that is mediated by the adaptor molecule myeloid differentiation factor 88 (MyD88).

TLR4 recognizes LPS present in the outer membrane of Gram-negative bacteria, signalling the host immune response and TLR5 recognizes Gram-negative and Gram-positive bacterial flagella (Abasht et al. 2008). This recognition of TLR4 occurs through macrophage recruitment (Beutler and Rietschel 2003) and subsequent mast cell degranulation with the release of inflammatory mediators and proteins that stimulate the immune system through the release of tumour necrosis factor (TNF- α) by macrophages, nuclear factor kappa B (NF- κ B) and cyclooxygenases (COX-2), besides the action of neutrophils at sites of infection (Beumer et al. 2003; Bates et al. 2007; Mussa et al. 2013).

The alkaline phosphatase enzyme has the ability to attenuate the LPS-mediated inflammatory response, probably by dephosphorylation of the lipid A portion present in LPS (Chen et al. 2011). Beumer et al. (2003) reported a difference in TNF- α release between pigs that were treated with LPS or LPS + IAP. Also, in this study, the authors observed that 80% of the tested mice survived the lethal infection of Gram-negative bacteria when treated with the IAP isoenzyme, suggesting that the enzyme action reduces the induction of the inflammatory response. Several studies have shown that

IAP activity (Koyama et al. 2002; Bates et al. 2007; Goldberg et al. 2008) and the expression of TLR4 and IAP (Abasht et al. 2008) increase in the presence of LPS, data that were also recently presented in studies developed by Melo et al. (2016).

However, LPS is not the only bacterial structure capable of inducing inflammatory response and is not the only target of IAP (Chen et al. 2010). Bacterial structures such as CpG-DNA and flagella are recognized in the host by TLR9 and TLR5 receptors, respectively and, as well as LPS, recognized by TLR4, can cause inflammatory response and induction of cytokine production (Shinkai et al. 2006; Tohno et al. 2006). IAP actions in dephosphorylation of CpG-DNA, bacterial flagella and LPS inhibit the induction of proinflammatory cytokine IL-8 mediated by the recognition of these bacterial structures by the host (Chen et al. 2010).

In addition, IAP also has the ability to dephosphorylate the nucleotide uridine diphosphate (UDP), a proinflammatory nucleotide involved in the inflammatory bowel disease (IBD) released by the host during inflammatory processes (Moss et al. 2013). The aforementioned authors also reported that UDP release increases the expression of P2Y₆ receptors, which have been presented as stimulants of IL-8 production. In the same way as IAP dephosphorylates UDP, the enzyme also promotes the growth of commensal organisms by dephosphorylation of the adenosine triphosphate (ATP) nucleotide. The presence of bowel luminal ATP inhibits the growth of commensal

bacteria; however, when dephosphorylated in adenosine diphosphate (ADP) and adenosine monophosphate (AMP), the inhibitory effect of bacterial growth was not observed (Malo et al. 2014).

IAP has the ability to recompose the gut commensal microbiota in dysbiosis situations, which are often factors related to early-weaned piglets, therapeutic treatment with AGP and the causes of bowel disease promoted by increased susceptibility of the host to opportunistic enteric pathogens such as *Salmonella enterica* serovar Typhimurium and *Clostridium difficile* (Alam et al. 2014). In the same study, the authors reported that the use of oral IAP promoted a decrease in inflammation in the colon, as evidenced by histology and blunted IL-1 β response.

IAP's function in cleaving ATP may also promote the maintenance of bowel homeostasis due to the effect of luminal ATP in stimulating the release of bicarbonate (HCO $_3^-$) by the enterocyte (Mizumori et al. 2009). In the absence of IAP, the HCO $_3^-$ secretion increases with ATP involvement via P2Y receptor activation (Akiba et al. 2007). However, in the presence of IAP, the accumulated ATP in the bowel lumen is dephosphorylated, regulating HCO $_3^-$ secretion. Thus, the IAP presents the protective function of the bowel mucosa by mediating HCO $_3^-$ secretion, maintaining homeostasis and preventing the cell injury due to local acidification, since bowel pH influences enzyme activity and expression.

IAP activity was found at pH 8 to 10 (Koyama et al. 2002) and pH 7.5 (Poelstra et al. 1997), while at pH 5 (Poelstra et al. 1997) and 2.2 (Akiba et al. 2007) it showed no enzymatic activity. Corroborating this idea, Akiba et al. (2007) reported that duodenal secretion of HCO $_3^-$ alkalinizes the microclimate surrounding IAP, increasing its activity, and found that L-cysteine inhibited IAP activity *in vitro* and *in vivo* through the production of hydrogen sulphide, and increased acid-induced duodenal HCO $_3^-$ secretion *in vivo*, confirming its effectiveness as an IAP inhibitor. Also, in this study, L-phenylalanine inhibited less *in vitro* IAP activity and partially increased acid-induced duodenal HCO $_3^-$ secretion, while the D-phenylalanine had no effect on the *in vitro* IAP activity and *in vivo* HCO $_3^-$ secretion.

Another effect of IAP is the minute-by-minute regulation of calcium (Ca $^{2+}$) absorption through bowel pH modulation, in which at alkaline pH its activity reduced bowel pH due to the enzyme con-

centration and luminal Ca $^{2+}$ content, and at low pH, the Ca $^{2+}$ absorption was reduced (Brun et al. 2014). This IAP effect on reducing pH reveals that a part of Ca $^{2+}$ absorption is independent of the vitamin D action and thus IAP acts by modulating the absorption of high Ca $^{2+}$ concentrations in the enterocyte, which could promote a potential toxic effect. In addition, Lowe and John (2018) reported that zinc and magnesium minerals are important IAP cofactors, that is, IAP activity and the induction of metabolic processes by IAP has a direct and indirect relationship with some minerals.

Studies have also been carried out on the potential therapeutic effect of IAP on IBD in humans using rats as an experimental model (Tuin et al. 2006; Martinez-Moya et al. 2012). Treatment of colitis with alkaline phosphatase in rats resulted in lower colon weight and macroscopic damage scores, besides to normalizing neutrophil marker expression (S100A8, LCN2 and IL-1 β) (Martinez-Moya et al. 2012). In addition, rats with colitis treated with alkaline phosphatase administered orally or rectally had lower bacterial translocation when compared to rats in the negative control and to rats that received antibiotics (Martinez-Moya et al. 2012). These data have demonstrated the beneficial effect of alkaline phosphatase in reducing the inflammatory response and risk of sepsis, which are evident when IAP activity is reduced by weaning (Lackeyram et al. 2010) or by a specific inhibitor (Martinez-Moya et al. 2012).

Other studies indicate that oral administration of IAP has beneficial effects in situations of severe intestinal epithelial injury, while in moderate inflammation endogenous IAP may be sufficient to counteract the aggravating effects of LPS. In addition, an approach that includes treatment with IAP has a therapeutic promise in case of severe IBD (Bol-Schoenmakers et al. 2010).

Corroborating these reports, Chen et al. (2011) mentioned that IAP is a defence factor of the bowel mucosa, a local immunomodulator, perhaps regulating the interaction of the LPS-TLR4 receptor between the commensal microbiota and the bowel epithelium. Finally, the IAP gene family has a strong evolutionary link with changes in GIT anatomy and food-induced microbial composition (Lalles 2014).

In short, deleterious effects on the bowel can be attributed to the reduction of IAP expression and its potential protective effect on post-weaning piglets, with diet being a factor capable of interfering

with the modulation of this enzyme (Goldberg et al. 2008). Thus, considering the physiological events that occur in piglets as a result of weaning, IAP can contribute positively to pig production when added via diet due to several benefits (Table 3).

Within this context, IAP can act as a host defence factor (Koyama et al. 2002), but a better understanding of the factors that regulate the expression of IAP and the promotion of intestinal health is necessary, with reduction of inflammatory processes, which may contribute to the treatment and prevention of IBD and, consequently, improve the health status and growth performance of animals. Finally, AGP used to be the most effective way to prevent PWD; however, with increasing bacterial resistance, alternatives to AGP are urgently needed. Thus, feed additives such as IAP can prevent enterotoxigenic *Escherichia coli*-associated post-weaning diarrhoea and improve the intestinal health of post-weaning piglets.

Conclusion

Research that verifies the additional effect of IAP on the piglet diets post challenge with ETEC is scarce and needs further investigation. Finally, ETEC strains are directly related to the post-weaning period, mainly to PWD events. IAP has a potential effect on the intestinal health of piglets, but it is not yet known to what extent its effect is validated because the intestinal health in this transition period is modulated by several stressors. So far, many alternatives have been investigated for AGP withdrawal, but this process may take a long time. There is not a single ingredient or product capable of resolving all intestinal health issues, but rather the association of several factors. The nutrition aspect of the piglet is as important as issues related to pre- and post-weaning management. Alternatives to AGP reported in this review are in constant research and it is possible that in future other studies and methods

Table 3. Properties of the intestinal alkaline phosphatase (IAP) enzyme

General and specific functions	References
IAP secretion in the basolateral membrane	Lalles (2014); Fawley and Gourlay (2016); Haarhaus et al. (2017)
Fatty acid absorption regulation	Mahmood et al. (2002); McConnell et al. (2009); Lalles (2014); Fawley and Gourlay (2016); Haarhaus et al. (2017)
Dephosphorylation of ATP, ADP and AMP molecules	Mizumori et al. (2009); Lalles (2014); Malo et al. (2014); Fawley and Gourlay (2016); Haarhaus et al. (2017)
Dephosphorylation of specific bacterial components including LPS, CpG DNA, Pam-3-Cys and flagellin	Chen et al. (2010); Moss et al. (2013); Lalles (2014); Fawley and Gourlay (2016); Hamarneh et al. (2017); Haarhaus et al. (2017)
Modulation of duodenal bicarbonate secretion and enteric surface pH	Akiba et al. (2007); Mizumori et al. (2009); Brun et al. (2014); Lalles (2014)
Reduction of intestinal bacterial translocation	Chen et al. (2011); Lalles (2014); Malo et al. (2014); Fawley and Gourlay (2016); Haarhaus et al. (2017); Bilski et al. (2017)
Intestinal tolerance and development of commensal bacteria	Lalles (2010); Lalles (2014); Malo et al. (2014); Bilski et al. (2017)
Control of local and systemic inflammation or infection	Beumer et al. (2003); Tuin et al. (2006); Bol-Schoenmakers et al. (2010); Ramasamy et al. (2011); Martinez-Moya et al. (2012); Moss et al. (2013); Alam et al. (2014); Lalles (2014); Fawley and Gourlay (2016); Bilski et al. (2017); Hamarneh et al. (2017)
Nucleotide dephosphorylation	Lalles (2014); Fawley and Gourlay (2016); Haarhaus et al. (2017)
IAP secretion in the apical membrane (microvilli)	Lalles (2014); Fawley and Gourlay (2016); Haarhaus et al. (2017)
Ca ²⁺ absorption regulation	Brun et al. (2014); Lalles (2014); Fawley and Gourlay (2016); Haarhaus et al. (2017)
Inorganic phosphate homeostasis	Sasaki et al. (2018)

ADP = adenosine diphosphate; AMP = adenosine monophosphate; ATP = adenosine triphosphate; IAP = intestinal alkaline phosphatase; LPS = lipopolysaccharide

will be developed with the purpose of evaluating mechanisms that have not yet been researched, such as the *in vivo* use of bioinspired synthetic peptides in toxins against bacteria and other pathogens and antimicrobial photodynamic inactivation.

Conflict of interest

The authors declare no conflict of interest.

REFERENCES

- Abasht B, Kaiser MG, Lamont SJ. Toll-like receptor gene expression in cecum and spleen of advanced intercross line chicks infected with *Salmonella enterica* serovar Enteritidis. *Vet Immunol Immunopathol*. 2008 Jun 15;123(3-4):314-23.
- Adewole DI, Kim IH, Nyachoti CM. Gut health of pigs: Challenge models and response criteria with a critical analysis of the effectiveness of selected feed additives – A review. *Asian-Australasian J Anim Sci*. 2016 Nov;29(7):909-24.
- Agustina H, Asyifa I, Aziz A, Hernowo BS. The role of osteocalcin and alkaline phosphatase immunohistochemistry in osteosarcoma diagnosis. *Patholog Res Int*. 2018 May 3;1-5.
- Akiba Y, Mizumori M, Guth PH, Engel E, Kaunitz JD. Duodenal brush border intestinal alkaline phosphatase activity affects bicarbonate secretion in rats. *Am J Physiol Gastrointest Liver Physiol*. 2007 Dec;293(6):G1223-33.
- Akira S, Takeda K, Kaisho T. Toll-like receptors: Critical proteins linking innate and acquired immunity. *Nat Immunol*. 2001 Aug;2(8):675-80.
- Alam SN, Yamine H, Moaven O, Ahmed R, Moss AK, Biswas B, Ghosh S. Intestinal alkaline phosphatase prevents antibiotic-induced susceptibility to enteric pathogens. *Ann Surg*. 2014 Apr;259(4):715-22.
- Anjos CM, Gois FD, Anjos CM, Rocha VS, Castro DES, Allaman IB, Silva FL, Carvalho PLO, Meneghetti C, Costa LB. Effects of dietary beta-glucans, glucomannans and mannan oligosaccharides or chlorohydroxyquinoline on the performance, diarrhea, hematological parameters, organ weight and intestinal health of weanling pigs. *Livest Sci*. 2019 May 1;223:39-46.
- Bates JM, Akerlund J, Mittge E, Guillemin K. Intestinal alkaline phosphatase detoxifies lipopolysaccharide and prevents inflammation in zebrafish in response to the gut microbiota. *Cell Host Microbe*. 2007 Dec 13;2(6):371-82.
- Barba-Vidal E, Roll VFB, Castillejos L, Guerra-Ordaz AA, Manteca X, Mallo JJ, Martin-Orue SM. Response to a *Salmonella* Typhimurium challenge in piglets supplemented with protected sodium butyrate or *Bacillus licheniformis*: Effects on performance, intestinal health and behavior. *Transl Anim Sci*. 2017a Apr 1;1(2):186-200.
- Barba-Vidal E, Castillejos L, Lopez-Colom P, Rivero Urgell M, Moreno Munoz JA, Martin-Orue SM. Evaluation of the probiotic strain *Bifidobacterium longum* subsp. *infantis* CECT 7210 capacities to improve health status and fight digestive pathogens in a piglet model. *Front Microbiol*. 2017b Apr 11;8:533.
- Barba-Vidal E, Martin-Orue SM, Castillejos L. Review: Are we using probiotics correctly in post-weaning piglets? *Animal*. 2018 Dec;12(12):2489-98.
- Bedford MR, Cowieson AJ. Exogenous enzymes and their effects on intestinal microbiology. *Anim Feed Sci Tech*. 2012 Apr 20;173(1-2):76-85.
- Beumer C, Wulferink M, Raaben W, Fiechter D, Brands R, Seinen W. Calf intestinal alkaline phosphatase, a novel therapeutic drug for lipopolysaccharide (LPS)-mediated diseases, attenuates LPS toxicity in mice and piglets. *J Pharmacol Exp Ther*. 2003 Nov;307(2):737-44.
- Beutler B, Rietschel ET. Innate immune sensing and its roots: The story of endotoxin. *Nat Rev Immunol*. 2003 Feb;3(2):169-76.
- Bischoff SC. “Gut health”: A new objective in medicine? *BMC Med*. 2011 Mar 14;9(1):24.
- Bilski J, Mazur-Bialy A, Wojcik D, Zahradnik-Bilska J, Brzozowski B, Magierowski M, Mach T, Magierowska K, Brzozowski T. The role of intestinal alkaline phosphatase in inflammatory disorders of gastrointestinal tract. *Mediators Inflamm*. 2017 Feb 21;1-9.
- Bol-Schoenmakers M, Fiechter D, Raaben W, Hassing I, Bleumink R, Kruijswijk D, Maijor K, Tersteeg-Zijderveld M, Brands R, Pieters R. Intestinal alkaline phosphatase contributes to the reduction of severe intestinal epithelial damage. *Eur J Pharmacol*. 2010 May 10;633(1-3):71-7.
- Brun LR, Brance ML, Lombarte M, Lupo M, Di Loreto VE, Rigalli A. Regulation of intestinal calcium absorption by luminal calcium content: Role of intestinal alkaline phosphatase. *Mol Nutr Food Res*. 2014 Apr;58(7):1546-51.
- Carabotti M, Scirocco A, Maselli MA, Severi C. The gut-brain axis: Interactions between enteric microbiota, central and enteric nervous systems. *Ann Gastroenterol*. 2015 Apr-Jun;28(2):203-9.
- Celi P, Cowieson AJ, Fru-Nji F, Steinert RE, Klunter AM, Verlhac V. Gastrointestinal functionality in animal nutrition and health: New opportunities for sustainable animal production. *Anim Feed Sci Technol*. 2017 Dec 1;234:88-100.
- Chen KT, Malo MS, Moss AK, Zeller S, Johnson P, Ebrahimi F, Mostafa G, Alam SN, Ramasamy S, Warren HS,

- Hohmann EL, Hodin RA. Identification of specific targets for the gut mucosal defense factor intestinal alkaline phosphatase. *Am J Physiol Gastrointest Liver Physiol*. 2010 Aug;299(2):467–75.
- Chen KT, Malo MS, Beasley-Topliffe LK, Poelstra K, Millan JL, Mostafa G, Alam SN, Ramasamy S, Warren HS, Hohmann EL, Hodin RA. A role for intestinal alkaline phosphatase in the maintenance of local gut immunity. *Dig Dis Sci*. 2011 Feb;56(4):1020–7.
- Cheng G, Hao H, Xie S, Wang X, Dai M, Huang L, Yuan Z. Antibiotic alternatives: The substitution of antibiotics in animal husbandry? *Front Microbiol*. 2014 May 13;5:1–16.
- David LA, Maurice CF, Carmody RN, Gootenberg DB, Button JE, Wolfe BE, Ling AV, Devlin AS, Varma Y, Fischbach MA, Biddinger SB, Dutton RJ, Turnbaugh PJ. Diet rapidly and reproducibly alters the human gut microbiome. *Nature*. 2014 Dec;505(7484):559–63.
- Dersjant-Li Y, Wealleans AL, Barnard LP, Lane S. Effect of increasing *Buttiauxella* phytase dose on nutrient digestibility and performance in weaned piglets fed corn or wheat based diets. *Anim Feed Sci Tech*. 2017 Dec 1;234:101–9.
- De Lange CFM, Pluske J, Gong J, Nyachoti CM. Strategic use of feed ingredients and feed additives to stimulate gut health and development in young pigs. *Livest Sci*. 2010 Sep 1;134(1–3):124–34.
- Duca FA, Sakar Y, Covasa M. The modulatory role of high fat feeding on gastrointestinal signals in obesity. *J Nutr Biochem*. 2013 Oct 1;24(10):1663–77.
- Fawley J, Gourlay DM. Intestinal alkaline phosphatase: A summary of its role in clinical disease. *J Surg Res*. 2016 May 1;202(1):225–34.
- Foster JA, Rinaman L, Cryan JF. Stress and the gut-brain axis: Regulation by the microbiome. *Neurobiol Stress*. 2017 Mar;7:124–36.
- Ghanim H, Abuaysheh S, Sia CL, Korzeniewski K, Chaudhuri A, Fernandez-Real JM, Dandona P. Increase in plasma endotoxin concentrations and the expression of toll-like receptors and suppressor of cytokine signaling-3 in mononuclear cells after a high-fat, high-carbohydrate meal: Implications for insulin resistance. *Diabetes Care*. 2009 Dec 1;32(12):2281–7.
- Goldberg RF, Austen WG, Zhang X, Munene G, Mostafa G, Biswas S, McCormack M, Eberlin KR, Nguyen JT, Tatlidede HS, Warren HS, Narisawa S, Millan JL, Hodin RA. Intestinal alkaline phosphatase is a gut mucosal defense factor maintained by enteral nutrition. *Proc Natl Acad Sci*. 2008 Mar 4;105(9):3551–6.
- Gu R, Sun Y. Does serum alkaline phosphatase level really indicate the prognosis in patients with osteosarcoma? A meta-analysis. *J Cancer Res Ther*. 2018 Jun 1;14(9):S468–72.
- Gul SS, Hamilton ARL, Munoz AR, Phupitakphol T, Liu W, Hyoju SK, Economopoulos KP, Morrison S, Hu D, Zhang W, Gharedaghi MH, Huo H, Hamarneh SR, Hodin RA. Inhibition of the gut enzyme intestinal alkaline phosphatase may explain how aspartame promotes glucose intolerance and obesity in mice. *Appl Physiol Nutr Metab*. 2017 Nov;42(1):77–83.
- Haarhaus M, Brandenburg V, Kalantar-Zadeh K, Stenvinkel P, Magnusson P. Alkaline phosphatase: A novel treatment target for cardiovascular disease in CKD. *Nat Rev Nephrol*. 2017 May;13(7):429–42.
- Hamarneh SR, Kim BM, Kaliannan K, Morrison SA, Tantillo TJ, Tao Q, Mohamed MMR, Ramirez JM, Karas A, Liu W, Hu D, Teshager A, Gul SS, Economopoulos KP, Bhan AK, Malo MS, Choi MY, Hodin RA. Intestinal alkaline phosphatase attenuates alcohol-induced hepatosteatosis in mice. *Dig Dis Sci*. 2017 Apr;62(8):2021–34.
- Han YS, Tang CH, Zhao QY, Zhan TF, Zhang K, Han YM, Zhang JM. Effects of dietary supplementation with combinations of organic and medium chain fatty acids as replacements for chlortetracycline on growth performance, serum immunity, and fecal microbiota of weaned piglets. *Livest Sci*. 2018 Oct 1;216:210–8.
- Heinrich D, Bruland O, Guise TA, Suzuki H, Sartor O. Alkaline phosphatase in metastatic castration-resistant prostate cancer: Reassessment of an older biomarker. *Futur Oncol*. 2018 Jun;14(24):2543–56.
- Honda K, Littman DR. The microbiota in adaptive immune homeostasis and disease. *Nature*. 2016 Jul;535(7610):75–84.
- Hu S, Cao X, Wu Y, Mei X, Xu H, Wang Y, Zhang X, Gong L, Li W. Effects of probiotic *Bacillus* as an alternative of antibiotics on digestive enzymes activity and intestinal integrity of piglets. *Front Microbiol*. 2018 Oct 22;9:1–9.
- Jayaraman B, Nyachoti CM. Husbandry practices and gut health outcomes in weaned piglets: A review. *Anim Nutr*. 2017 Sep 1;3(3):205–11.
- Kelly JR, Kennedy PJ, Cryan JF, Dinan TG, Clarke G, Hyland NP. Breaking down the barriers: The gut microbiome, intestinal permeability and stress-related psychiatric disorders. *Front Cell Neurosci*. 2015 Oct 14;9:1–20.
- Kiarie E, Romero LF, Nyachoti CM. The role of added feed enzymes in promoting gut health in swine and poultry. *Nutr Res Rev*. 2013 Jun;26(1):71–88.
- Kil DY, Kwon WB, Kim BG. Dietary acidifiers in weanling pig diets: A review. *Ver Colom Cienc Pecua*. 2011 Jul-Sep;24(3):231–47.
- Kogut MH, Arsenault RJ. Gut health: The new paradigm in food animal production. *Front Vet Sci*. 2016 Aug 31;3:10–3.
- Koyama I, Matsunaga T, Harada T, Hokari S, Komoda T. Alkaline phosphatases reduce toxicity of lipopolysaccha-

- rides in vivo and in vitro through dephosphorylation. *Clin Biochem.* 2002 Sep 1;35(6):455-61.
- Lackeyram D, Yang C, Archbold T, Swanson KC, Fan MZ. Early weaning reduces small intestinal alkaline phosphatase expression in pigs. *J Nutr.* 2010 Jan;140(3):461-8.
- Lalles JP. Intestinal alkaline phosphatase: Multiple biological roles in maintenance of intestinal homeostasis and modulation by diet. *Nutr Rev.* 2010 Jun 1;68(6):323-32.
- Lalles JP. Intestinal alkaline phosphatase: Novel functions and protective effects. *Nutr Rev.* 2014 Feb 1;72(2):82-94.
- Li Q, Gabler NK, Loving CL, Gould SA, Patience JF. A dietary carbohydrase blend improved intestinal barrier function and growth rate in weaned pigs fed higher fiber diets. *J Anim Sci.* 2018 Oct;96(12):5233-43.
- Liao SF, Nyachoti M. Using probiotics to improve swine gut health and nutrient utilization. *Anim Nutr.* 2017 Dec 1;3(4):331-43.
- Liu Y, Espinosa CD, Abelilla JJ, Casas GA, Lagos LV, Lee SA, Kwon WB, Mathai JK, Navarro DMDL, Jaworski NW, Stein HH. Non-antibiotic feed additives in diets for pigs: A review. *Anim Nutr.* 2018 Jun 1;4(2):113-25.
- Long SF, Xu YT, Pan L, Wang QQ, Wang CL, Wu JY, Wu YY, Han YM, Yun CH, Piao XS. Mixed organic acids as antibiotic substitutes improve performance, serum immunity, intestinal morphology and microbiota for weaned piglets. *Anim Feed Sci Tech.* 2018 Jan 1;235:23-32.
- Lowe D, John S. Alkaline phosphatase. *StatPearls* [Internet]. 2018 Apr [cited 2019 Dec 1]. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK459201/>.
- Lu H, Kuhn I, Bedford MR, Whitfield H, Brearley C, Adeola O, Ajuwon KM. Effect of phytase on intestinal phytate breakdown, plasma inositol concentrations, and glucose transporter type 4 abundance in muscle membranes of weanling pigs. *J Anim Sci.* 2019 Jul;97(9):3907-19.
- Mahmood A, Engle MJ, Alpers DH. Secreted intestinal surfactant-like particles interact with cell membranes and extracellular matrix proteins in rats. *J Physiol.* 2002 Jul;542(1):237-44.
- Malo MS, Moaven O, Muhammad N, Biswas B, Alam SN, Economopoulos KP, Gul SS, Hamarneh SR, Malo NS, Teshager A, Mohamed MMR, Tao Q, Narisawa S, Millan JL, Hohmann EL, Shaw Warren H, Robson SC, Hodin RA. Intestinal alkaline phosphatase promotes gut bacterial growth by reducing the concentration of luminal nucleotide triphosphates. *Am J Physiol Gastrointest Liver Physiol.* 2014 May 15;306(10):826-38.
- Martinez-Moya P, Ortega-Gonzalez M, Gonzalez R, Anzola A, Ocon B, Hernandez-Chirlaque C, Lopez-Posadas R, Suarez MD, Zarzuelo A, Martinez-Augustin O, Sanchez de Medina F. Exogenous alkaline phosphatase treatment complements endogenous enzyme protection in colonic inflammation and reduces bacterial translocation in rats. *Pharmacol Res.* 2012 Aug;66(2):144-53.
- McConnell RE, Higginbotham JN, Shifrin DA, Tabb DL, Coffey RJ, Tyska MJ. The enterocyte microvillus is a vesicle-generating organelle. *J Cell Biol.* 2009 Jun 29;185(7):1285-98.
- Melo ADB, Silveira H, Luciano FB, Rade C, Costa LB, Rostagno MH. Intestinal alkaline phosphatase: Potential roles in promoting gut health in weanling piglets and its modulation by feed additives – A review. *Asian-Australas J Anim Sci.* 2016 Jan;29(1):16-22.
- Mizumori M, Ham M, Guth PH, Engel E, Kaunitz JD, Akiba Y. Intestinal alkaline phosphatase regulates protective surface microclimate pH in rat duodenum. *J Physiol.* 2009 Jul 15;587(14):3651-63.
- Moeser AJ, Pohl CS, Rajput M. Weaning stress and gastrointestinal barrier development: Implications for lifelong gut health in pigs. *Anim Nutr.* 2017 Dec;3(4):313-21.
- Moreira APB, Texeira TFS, Ferreira AB, Peluzio MCG, Alfenas RCG. Influence of a high-fat diet on gut microbiota, intestinal permeability and metabolic endotoxaemia. *Br J Nutr.* 2012 Sep;108(5):801-9.
- Moss AK, Hamarneh SR, Mohamed MMR, Ramasamy S, Yammine H, Patel P, Kaliannan K, Alam SN, Muhammad N, Moaven O, Teshager A, Malo NS, Narisawa S, Millan JL, Shaw Warren H, Hohmann E, Malo MS, Hodin RA. Intestinal alkaline phosphatase inhibits the proinflammatory nucleotide uridine diphosphate. *Am J Physiol Gastrointest Liver Physiol.* 2013 Mar 15;304(6):G597-604.
- Mukhopadhyaya A, O'Doherty JV, Sweeney T. A combination of yeast beta-glucan milk hydrolysate is a suitable alternative to zinc oxide in the race to alleviate post-weaning diarrhoea in piglets. *Sci Rep.* 2019 Jan 24;9(1):1-11.
- Mussa T, Ballester M, Silva-Campa E, Baratelli M, Busquets N, Lecours MP, Dominguez J, Amadori M, Fraile L, Hernandez J, Montoya M. Swine, human or avian influenza viruses differentially activates porcine dendritic cells cytokine profile. *Vet Immunol Immunopathol.* 2013 Jul 15;154(1-2):25-35.
- Pan L, Zhao PF, Ma XK, Shang QH, Xu YT, Long SF, Wu Y, Yuan FM, Piao XS. Probiotic supplementation protects weaned pigs against enterotoxigenic *Escherichia coli* k88 challenge and improves performance similar to antibiotics. *J Anim Sci.* 2017 Jun;95(6):2627-39.
- Pearlin BV, Muthuvel S, Govidasamy P, Villavan M, Alagawany M, Ragab Farag M, Dhama K, Gopi M. Role of acidifiers in livestock nutrition and health: A review. *J Anim Physiol Anim Nutr.* 2020 Jan;104(2):558-69.
- Peng M, Wang Z, Peng S, Zhang M, Duan Y, Li F, Shi S, Yang Q, Zhang C. Dietary supplementation with the extract from *Eucommia ulmoides* leaves changed epithelial

- restitution and gut microbial community and composition of weanling piglets. *PLoS One*. 2019 Sep 26;14(9): e0223002.
- Peeters L, Mostin L, Wattiau P, Boyen F, Dewulf J, Maes D. Efficacy of *Clostridium butyricum* as probiotic feed additive against experimental *Salmonella Typhimurium* infection in pigs. *Livest Sci*. 2019 Mar 1;221:82-5.
- Pluske JR, Turpin DL, Kim JC. Gastrointestinal tract (gut) health in the young pig. *Anim Nutr*. 2018 Jun;4(2):187-96.
- Poelstra K, Bakker WW, Klok PA, Kamps JAAM, Hardonk MJ, Meijer DKE. Dephosphorylation of endotoxin by alkaline phosphatase in vivo. *Am J Pathol*. 1997 Oct;151(4): 1163-9.
- Rader BA. Alkaline phosphatase, an unconventional immune protein. *Front Immunol*. 2017 Aug 3;8:1-6.
- Ramasamy S, Nguyen DD, Eston MA, Alam SN, Moss AK, Ebrahimi F, Biswas B, Mostafa G, Chen KT, Kaliannan K, Yammine H, Narisawa S, Millan JL, Warren HS, Hohmann EL, Mizoguchi E, Reinecker HC, Bhan AK, Snapper SB, Malo MS, Hodin RA. Intestinal alkaline phosphatase has beneficial effects in mouse models of chronic colitis. *Inflamm Bowel Dis*. 2011 Jul;17(2):532-42.
- Sasaki S, Segawa H, Hanazaki A, Kirino R, Fujii T, Ikuta K, Noguchi M, Sasaki S, Koike M, Tanifuji K, Shiozaki Y, Kaneko I, Tatsumi S, Shimohata T, Kawai Y, Narisawa S, Millan JL, Miyamoto KI. A role of intestinal alkaline phosphatase 3 (Akp3) in inorganic phosphate homeostasis. *Kidney Blood Press Res*. 2018 Sep;43(5):1409-24.
- Schiering C, Krausgruber T, Chomka A, Frohlich A, Adelman K, Wohlfert EA, Pott J, Griseri T, Bollrath J, Hegazy AN, Harrison OJ, Owens BMJ, Lohning M, Belkaid Y, Fallon PG, Powrie F. The alarmin IL-33 promotes regulatory T-cell function in the intestine. *Nature*. 2014 Jul; 513(7519):564-8.
- Shinkai H, Tanaka M, Morozumi T, Eguchi-Ogawa T, Okumura N, Muneta Y, Awata T, Uenishi H. Biased distribution of single nucleotide polymorphisms (SNPs) in porcine Toll-like receptor 1 (TLR1), TLR2, TLR4, TLR5, TLR6 genes. *Immunogenetics*. 2006 Apr;58(4):324-30.
- Song ZH, Xiao K, Ke YL, Jiao LE, Hu CH. Zinc oxide influences mitogen-activated protein kinase and TGF- β 1 signaling pathways, and enhances intestinal barrier integrity in weaned pigs. *Innate Immun*. 2015 Jun;21(4):341-8.
- Stevanovic ZD, Bosnjak-Neumuller J, Pajic-Lijakovic I, Raj J, Vasiljevic M. Essential oils as feed additives – Future perspectives. *Molecules*. 2018 Jul 14;23(7):1-20.
- Suryanarayana MVAN, Durga S. Role of phyto-genic feed additives in swine production – A review. *Int J Environ Agric Biotechnol*. 2018 May-Jun;3(3):1071-8.
- Sussman NL, Eliakim R, Rubin D, Perlmutter DH, Deschryver-Kecskemeti K, Alpers DH. Intestinal alkaline phosphatase is secreted bidirectionally from villous enterocytes. *Am J Physiol Gastrointest Liver Physiol*. 1989 Jul 1;257(1):G14-23.
- Takeda K, Kaisho T, Akira S. Toll-like receptors. *Annu Rev Immunol*. 2003 Jan;21(1):335-76.
- Takeda K, Akira S. TLR signaling pathways. *Semin Immunol*. 2004 Feb;16(1):3-9.
- Tan P, Xie N, Ai J, Xu H, Xu H, Liu L, Yang L, Wei Q. The prognostic significance of Albumin-to-Alkaline Phosphatase Ratio in upper tract urothelial carcinoma. *Sci Rep*. 2018 Aug 17;8(1):1-8.
- Tian S, Wang J, Yu H, Wang J, Zhu W. Effects of galactooligosaccharides on growth and gut function of newborn suckling piglets. *J Anim Sci Biotechnol*. 2018 Oct;9(1):1-11.
- Tohno M, Shimosato T, Moue M, Aso H, Watanabe K, Kawai Y, Yamaguchi T, Saito T, Kitazawa H. Toll-like receptor 2 and 9 are expressed functional in gut-associated lymphoid tissues of presuckling newborn swine. *Vet Res*. 2006 Nov-Dec;37(6):791-812.
- Tuin A, Huizinga-Van Der Vlag A, Van Loenen-Weemaes AMMA, Meijer DKE, Poelstra K. On the role and fate of LPS-dephosphorylating activity in the rat liver. *Am J Physiol Gastrointest Liver Physiol*. 2006 Feb;290(2): G377-85.
- Upadhaya SD, Lee KY, Serpunja S, Song TH, Kim IH. Growth performance, nutrient digestibility, fecal microbiota and fecal noxious gas emission in weaning pigs fed high- and low-density diet with and without protected organic acid blends. *Anim Feed Sci Tech*. 2018 May 1; 239:1-8.
- Wei HK, Xue HX, Zhou ZX, Peng J. A carvacrol-thymol blend decreased intestinal oxidative stress influenced selected microbes without changing the messenger RNA levels of tight junction proteins in jejunal mucosa of weaning piglets. *Animal*. 2017 Feb;11(2):193-201.
- Werling D, Jungi TW. TOLL-like receptors linking innate adaptive immune response. *Vet Immunol Immunopathol*. 2003 Jan 10;91(1):1-12.
- Xu YT, Liu LI, Long SE, Pan L, Piao XS. Effect of organic acids and essential oils on performance, intestinal health and digestive enzyme activities of weaned pigs. *Anim Feed Sci Tech*. 2018 Jan;235:110-9.
- Yeoman CJ, White BA. Gastrointestinal tract microbiota and probiotics in production animals. *Annu Rev Anim Biosci*. 2014 Feb;2:469-86.
- Zhai H, Liu H, Wang S, Wu J, Klueenter AM. Potential of essential oils for poultry and pigs. *Anim Nutr*. 2018 Jun; 4(2):179-86.

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