Intestinal alkaline phosphatase: a new gut health biomarker

Weaning is a stressful event for young piglets: in general, feed intake and weight gain decrease, while the diarrhoea rate increases. Some ingredients and additives are supplied in piglets' diets during the post-weaning period in order to improve growth performance, generally through adjusting gut health.

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In order to evaluate their efficacy, some biomarkers are measured, or are under investigation. Recent discoveries showed that intestinal alkaline phosphatase (IAP) could be an interesting gut health biomarker.

What is IAP?

In a multitude of organisms, alkaline phosphatases (AP) play the role of dephosphorylating compounds, including ATP. These metalloenzymes are generally homodimeric proteins, with monomer containing cysteines residues, Zn2+ and Mg2+ ions. They can be non-specific (TNAP) and found in various tissues (bone, liver) or specific: in the placenta (PLAP), in the germline cells (GCAP) or in the intestinal epithelial cells (IAP).

IAP is synthesised in the cell and exported to the apical membrane of villus-associated enterocytes, all along the small intestine, then released into the intestinal lumen, and also into the bloodstream for a fraction, through basolateral secretion. Its expression and/or enzyme activity is regulated by many factors: developmental stage of the animal, feed intake, specific nutrients and other dietary compounds. IAP expression and activity decrease during starving and increase with refeeding, and with fat intake. Intestinal microbiota, including pathogenic E. coli, also stimulate IAP secretion, for example, during gut colonisation; this effect was demonstrated in germ-free zebrafish and may also be true in mammals. IAP plays various roles in the gastrointestinal tract; many biological functions have been discovered in recent years.

What are the roles of IAP?

- **Regulation of intestinal pH at the surface of enterocytes**

When the pH in the duodenum is too low, ATPase activity of IAP is low and an extracellular accumulation of ATP secreted by enterocytes occurs. This stimulates P2Y1 receptors and leads to secretion of bicarbonate, which neutralises duodenal surface pH. So IAP protects indirectly the intestinal mucosa against acid and pepsin-rich gastric chyme.

- **Detoxification of bacterial lipopolysaccharides (LPS)**

Lipopolysaccharides (LPS) are located in Gram-negative bacteria and in some Gram-positive bacteria. They contain three parts: O antigen, core oligosaccharide and lipid A (Fig. 1). Their toxicity resides in diphosphoryl-lipid A moiety which actives the Toll Like Receptor 4 (TLR4). This receptor takes part in pathogen recognition and can lead to the production of inflammatory cytokines (TNF, IL-1β).

IAP controls the inflammation and reduces the risk of sepsis by dephosphorylating lipid A moiety. It converts the diphosphoryl lipid A in monophosphoryl form, which is unable to bind to TLR4. This role was initially demonstrated in vitro, then in endothelial cells and in the zebrafish larvae. In addition, the anti-inflammatory effect of IAP was shown by exogenous IAP (bovine) administration in rats.

This potent anti-inflammatory property of IAP strongly contributes to prevent (or treat) metabolic disorders and obesity; IAP could also limit fat absorption.

- **Control of the gut microbiota**

IAP activity depends on intestinal bacteria and bacterial products and, in turn, influences the balance in microbial populations. For example, IAP could help improve intestinal disorders by contributing to restore the normal commensal bacteria balance.

- **Direct and indirect effects on intestinal mucosa permeability**

In addition to its various roles in the gut (regulation of intestinal pH, detoxification of bacterial LPS, action on intestinal microbiota, reduction of inflammation), IAP has a direct effect on the intestinal mucosa integrity through the regulation of the expression of some tight junctions' proteins. It can consequently reduce transmucosal passage of bacteria and risks of septic shock.

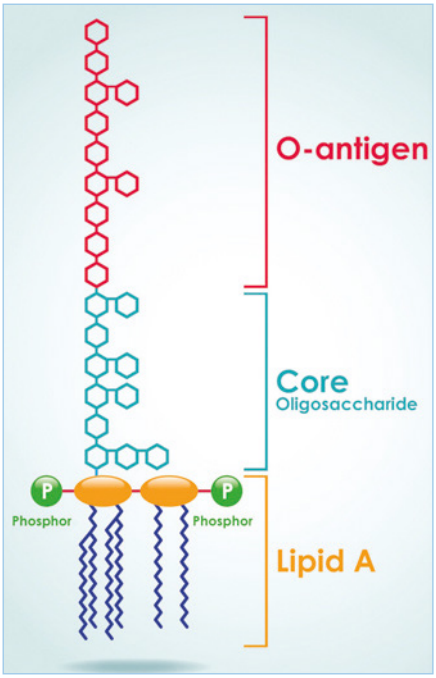

Fig. 1. LPS molecule.

Effect of Zn on IAP

Intestinal mucosa permeability of piglets increases during the post-weaning period. Compared to suckling piglets, weaned piglets showed a reduction of IAP abundance.

Feed ingredients can impact IAP expression and/or activity: for example, fat-rich diets and carbohydrates like starch and cellulose can increase IAP activity. Some studies also showed an effect of some additives: organic acids, essential oils, minerals.

In particular, it was observed that Zn supplementation stimulated IAP secretion; in addition, an increase of IAP activity was recorded in piglets fed a pharmacological dosage of zinc oxide (ZnO, 2,500ppm Zn),

Continued on page 12

Continued from page 11
compared to the group fed a negative control with Zn at a nutritional dosage.

ZnO is commonly used as a growth-promoter in piglet diets of non-EU countries and in medicated feeds of EU countries. When supplied at pharmacological dosage in weaned piglet diets, it can increase weight gain and reduce diarrhoea. Its mode of action is not fully elucidated, but its effects on gut health have been shown in various studies.

In a recent trial performed at Ghent University, the effect of ZnO on gut health was studied in experiments with different treatments: standard ZnO at nutritional (110ppm of supplemented Zn) or at pharmacological (2,400ppm of supplemented Zn) dosage and potentiated ZnO (HiZox) at two dosages (110 or 220ppm of supplemented Zn).

Intestinal transepithelial electrical resistance (TEER), measured in Ussing chambers, was chosen as a marker of intestinal mucosal integrity: a high TEER corresponds with a low intestinal permeability. TEER and IAP mRNA expression increased with pharmacological dosage of ZnO and potentiated ZnO, compared to standard ZnO at a nutritional dosage (Fig. 2).

These results were associated with other markers of gut health, including the reduction of intestinal E. coli. The effect of the potentiated ZnO source on IAP gene expression and on IAP activity was confirmed in other studies.

Conclusion

IAP plays various roles in the intestine of animals, such as microbiota regulation, detoxification of LPS, and direct and indirect effects on the integrity of the intestinal mucosa. Thus, it has positive effects on weaned piglets' intestine.

As IAP secretion is stimulated by Zn supplementation, an increase in IAP expression and/or activity could explain the positive effect of supplemental Zn on gut health and growth performance in piglets.

Therefore, IAP can be used as a gut health biomarker and validate the efficiency of Zn sources used in piglet feeds. ■

Fig. 2. TEER and alkaline phosphatase gene expression, according to experimental diets.