PROTECTIVE EFFECTS OF β GALACTOMANNANS OF VEGABLE ORIGIN ON THE INTESTINAL BARRIER FUNCTION

Javier Estévez and Sergi Carné
Industrial Técnica Pecuaria, S.A. (ITPSA)
Technical and Innovation Department

The intestinal epithelium is exposed to various components in the intestinal lumen, including toxins, microorganisms and antigens of different origins, among others.

The intestinal barrier function is a combination of elements that regulate the transport of macromolecules or antigens towards the internal environment through the intestinal epithelium. This ensures the maintenance of physiological functions (absorption of nutrients, secretion, etc.) to guarantee homeostasis.

Adequate barrier function prevents the uncontrolled passage of antigens and microorganisms in between enterocytes, thereby preventing excessive contact with the components of the enteral immune system and activation of inflammatory responses which would have a detrimental effect on intestinal health and on the absorption of nutrients from the diet.

WHAT is the INTESTINAL BARRIER FUNCTION?

The intestinal barrier is the body’s first line of defense and helps to fight infectious diseases that would otherwise compromise the health status of farms and would reduce productive parameters.
The intestinal barrier function comprises different levels of protection.

**COMPONENTS of the INTESTINAL BARRIER FUNCTION**

The intestinal contents are continuously surveilled by antimicrobial peptides, immunoglobulins, and digestive enzymes produced by the animal. In addition, protection is conferred by the microorganisms of the intestinal microbiota and their antimicrobial substances.

Another component is intestinal mucus, secreted by epithelial goblet cells, which forms a protective layer over the epithelium, helping to prevent adhesion and penetration of bacteria toward the internal environment.

More internally, the cylindrical monolayer epithelium which forms the intestinal mucosa is a physical barrier in itself, made up of enterocytes bound to one another by adhesion complexes including tight junctions (TJs), adherens junctions, desmosomes, and gap junctions.

Tight junctions are multi-protein structures that play a key role in the regulation of the intestinal barrier function. Tight junctions regulate the passage of ions and macromolecules through the paracellular space, i.e. the space between enterocytes.

Tight junctions are formed by transcellular proteins such as occludins, claudins, and JAM proteins, as well as intracellular proteins such as zona occludens (ZO) proteins, including ZO 1, ZO 2, and ZO 3 (Figure 1).

ZO proteins join transcellular proteins with an actomyosin ring from the enterocyte. The enterocyte contracts by the action of kinases such as myosin light-chain kinase (MLCK), which is activated by a number of stimuli, thereby opening the paracellular space and resulting in increased epithelial permeability.

Many pathogenic bacteria cause a disturbance of the epithelial barrier in order to facilitate intestinal colonization.

**EFFECTS of ENTEROBACTERIA ON EPITHELIAL BARRIER FUNCTION**

Certain pathogenic strains of *Escherichia coli* cause ZO-1 and actin filaments in the cytoskeleton to redistribute, which disrupts the structure of the tight junction.

*Salmonella typhimurium*, in addition to causing changes in the distribution of ZO-1, activates mechanisms to contract the apical pole of the cell (Brufau et al., 2015; Sears, 2000). This results in increased epithelial permeability and facilitates invasion by the pathogen, which leads to states of inflammation which can persist because of the increased contact of the immune system with the antigens in the intestinal contents.

This results in an increase in proinflammatory cytokines, which, in turn, have detrimental effects on the intestinal epithelial barrier function, thereby creating a vicious circle.
The mode of action of HβGMs has been studied by Badia et al. (2012), who conducted a number of studies on intestinal cell cultures in the presence and absence of Salmonella typhimurium (Figure 2).

The presence of HβGMs at 0.5 µg/mL significantly inhibited intestinal cell invasion by Salmonella, with approximately 60% inhibition versus the positive control. At HβGM levels of 10 µg/mL, the reduction was above 70% (Badia, Brufau et al., 2012).

These authors also evaluated the effect of HβGMs on the intestinal invasion capacity of E. coli K88 (Badia, Zanello et al., 2012) in intestinal cells protected with different levels of HβGMs, of up to 20 µg/mL.

A reduction in cellular invasion of up to 80% was observed due to the presence of β galactose-sensitive adhesins in E. coli (Figure 3).

Brufau et al. (2015) showed that HβGMs in the diet of chickens increased the secretion of intestinal mucus by goblet cells, not only compared to animals inoculated with 10^8 CFUs of Salmonella enterica serovar Enteritidis, but also compared to non-inoculated controls.

In addition, Brufau et al. reported a higher number of goblet cells, as assessed by electron microscopy, in animals with HβGMs included in their diet, and a lower number of bacteria adhered to the intestinal epithelium (Figure 4). A thicker layer of mucus, promoted by the addition to the diet of HβGMs, could make it more difficult for Salmonella spp., as well as other pathogens, to adhere to the epithelium, without inducing disruptive changes in the absorption of nutrients or affecting the productive parameters of animals (Brufau et al., 2015).

This would facilitate the fecal excretion of pathogens, which are no longer infective once bound to HβGMs, and would reduce the risk of infection.

The reduced pathogen-epithelium interaction is also demonstrated in this study by Brufau et al. In conditions of infection by Salmonella spp. and other species, several authors have reported an increase in the number of epithelial M (microfold) cells (Frost, Bland & Wallis, 1997; Jepson & Clark, 1998; Frost, 1997).

Hydrolyzed vegetable β-galactomannans (HβGMs) primarily act by blocking the fimbriae of enterobacteria

Since the European Union banned the use of antibiotics as growth promoters in 2006, a number of strategies have been developed to promote intestinal health and achieve good health status for animals and improved animal production levels.

In this regard, highly useful additives have been developed over the last few years, such as hydrolyzed vegetable β-galactomannans (HβGMs). HβGMs primarily act by blocking the fimbriae of enterobacteria, including Salmonella enterica and enterotoxigenic Escherichia coli (ETEC). Fimbriae are one of the bacterium’s main mechanisms by which it adheres to the epithelium, a key step in the process of colonization (Sharon & Lis, 1993).
M cells are responsible for transport of intestinal antigens to the gut-associated lymphoid tissue (GALT) and also for the passage of *Salmonella* through the intestinal wall.

M cells are responsible for transport of intestinal antigens to the gut-associated lymphoid tissue (GALT) by using lamellipodia, which are projections of the cell membrane linked to pathogen uptake processes performed by the cell.

M cells also contribute to the passage of *Salmonella* through the intestinal wall after they adhere using their fimbriae.

Therefore, the fact that the tissues of animals consuming HβGMs show a lower number of epithelial M cells and lower formation of lamellipodia indicates lower interaction of *Salmonella enterica* with the intestinal epithelium (Figure 3).

Furthermore, as seen above, in conditions of intestinal inflammation, tight junctions can become disrupted, compromising the integrity of the intestinal barrier function.

*Brufau et al.* (2015) observed, using confocal microscopy, that animals infected with *Salmonella enterica* show delocalization of tight junction (ZO) protein 1 (ZO-1) in conditions of intestinal inflammation. However, these effects of *Salmonella* are prevented in animals that consume β-galactomannans. These animals show a distribution of ZO-1 which corresponds to the actual position of TJs (Figure 3).

In the reports by Badia et al. (2012 and 2014), challenge of porcine intestinal cell (IPI-21) cultures with *Salmonella typhimurium* induced an increase in the gene expression (mRNA) of proinflammatory cytokines (IL-6, IL-1α, TNFα, and GM-CSF) and chemokines (CXCL-2, CXCL-8, and CXCL-10; CCL-2 and CCL-10) by epithelial cells.

In cultures of porcine dendritic cells, *Salmonella typhimurium* also increased gene expression (mRNA) of certain proinflammatory (TNFα, IL-6 and GM-CSF) and anti-inflammatory cytokines (IL-10), as well as a number of chemokines (CXCL-8 and CCL-17) (*Badia et al.*, 2012).

**Figure 5.** Confocal microscopy images with immunocolocalization of ZO-1 in chicken ilea. NC: non-inoculated chickens; PC: inoculated chickens; SA: inoculated chickens + 1 g/kg SALMOSAN (Industrial Técnica Pecuaria, S.A.); Cy: cytosol. (*Brufau et al.*, 2015).

**Figure 6.** Effect of hydrolyzed vegetable β-galactomannans (10 µg/mL, SALMOSAN, Industrial Técnica Pecuaria, S.A.) over mRNA expression of proinflammatory cytokines (TNFα and IL-6) in intestinal epithelial cell cultures co-cultured with *Salmonella*. Different letters indicate significant differences (p < 0.05; n = 6) (*Badia et al.*, 2012).
HβGMs can induce changes in certain toll-like receptors (TLRs), which play key roles in the activation and regulation of the immune response.

Unlike the observations in epithelial cells, in the presence of the bacteria, HβGMs led to a greater increase in the expression of proinflammatory cytokines TNFα and GM-CSF and anti-inflammatory cytokine IL-10 (p < 0.05) by dendritic cells, with no changes in the expression of IL-6 or CCL-17.

In the absence of Salmonella typhimurium in the culture, HβGMs induced the expression of TNFα and GM-CSF. Together with the results in porcine (IPI-21) enterocytes, this suggests that HβGMs could behave as potential immunomodulators per se.

On the other hand, in assays conducted at Research Institute IRTA (Institut de Recerca i Tecnologia Agroalimentàries), in which chickens and pigs were fed a HβGM-based commercial product, HβGMs were found to induce changes in certain toll-like receptors (TLRs).

TLRs are essential for activation and regulation of the immune response, as they recognize pathogen-associated molecular patterns (PAMPs), such as the lipopolysaccharide (LPS) present on Gram-negative bacteria (recognized by TLR-4), the lipoteichoic acid in the wall of Gram-positive bacteria (detected by TLR-2) or components of the flagella of certain bacteria, such as Salmonella spp. (flagellin recognized by TLR-5).

These changes in the expression levels of TLRs also have a positive impact on the intestinal barrier function, as increased activation of TLR-2 has been associated with an improvement in barrier function through changes in the distribution of ZO-1 (Caio, Gerken & Podalsky, 2004).

In addition, the decreased expression of TLR-5 can also be seen as a reduced interaction with its natural agonist flagellin, present in the flagella of pathogens such as Salmonella typhimurium, as a result of pathogen elimination following its uptake by hydrolyzed βGMs.

Conversely, the expression of TLR-5, which was higher in pigs inoculated with S. typhimurium, decreased when animals took HβGMs in the diet, reaching levels similar to those seen in non-inoculated animals.
The integrity of the intestinal barrier function is essential to preserve the animal’s health, reducing the risk of infectious disease and contributing to the adequate maintenance of physiological intestinal functions.

Hydrolyzed vegetable β-galactomannans bind to the fimbriae of enterobacteria such as *Salmonella* spp. and *Escherichia coli*, blocking their mechanism of epithelial adhesion and preventing colonization.

Hydrolyzed vegetable β-galactomannans have an enhancing effect on the intestinal barrier function in conditions of pathogen exposure.

Hydrolyzed vegetable β-galactomannans have an immunomodulatory effect both in conditions of pathogen exposure and in baseline conditions.