Algae bioactive compounds and their impact on the early development of the piglet's immune response.

- ## Contextualization-Introduction

The strong level of demand in animal production means that piglets must adapt their gastrointestinal tract in a few weeks to move from a liquid to a solid diet. The gut of piglets before birth lacks microbes, but it quickly passes from this initial state, free of germs, to a state of extremely dense microbial population, which suffers a microbial succession and establishes a microbial community similar to that of adults (Guevara et al., 2019). All these factors and the growing concern about the restriction of the use of antimicrobials lead to preventive and stabilizing strategies of intestinal function that should be implemented as the main point at the farm level. Hence, the search for nutritional strategies that have a high acceptance for the newborn piglet and that favor the rapid and adequate development of the gastrointestinal tract and its microbial composition, are showing a growing interest for modern producers. In this context, **Igusol Advance S.A has developed GreenTonic, as an invigorating solution that improves the survival, intestinal stability and initial development of the piglet, and, at the same time, stimulates the voluntary consumption of feed and its post-weaning adaptation.**

To obtain a complex and complete response to the challenges faced by the piglet in its first months of life, **GreenTonic** presents a wide range of **natural components** that seek to maximize the productive response. For this, the immune and gastrointestinal development of the piglet is stimulated, while the bacterial flora is balanced and high digestibility compounds are included to maximize the homogeneous development of the litter.

The **GreenTonic** natural solution has a careful composition since, among other components, it contains amino acids with high digestibility and nutritional value, flavorings that stimulate voluntary consumption, free organic acids and their salts that increase enzyme activation. Moreover, the presence of bioactive compounds from algae is noteworthy, which as we will analyze below, it has been seen that the algae present a complex composition of polyphenols, polysaccharides, fatty acids, phytosterols and carotenoids that have been linked to strong antioxidant and immunostimulating powers.

- ## Piglet immune response

The importance of a good intake of **colostrum** in the first hours of life is crucial for t**he survival of the piglet**, providing a complete immune response to defend against the first external aggressions. As it is observed in the **figure 1**, the peak in immunity acquired with colostrum or passive immunity occurs at 7 days of life of the piglet. However, after this first immunological challenge, the moment of the fall of **acquired immunity and the beginning of the development of active or own immunity** supposes a second turning point, which is also associated with another crucial moment in the life of the piglet; **the weaning**. In fact, the piglet's active immunity is not fully developed until at least 2 weeks after weaning. Therefore, during weaning, in addition to the social, nutritional and environmental factors, we also find **a window on the immunity** and protection of the piglet. Therefore, we could say that weaning conditions are in place for a perfect storm.

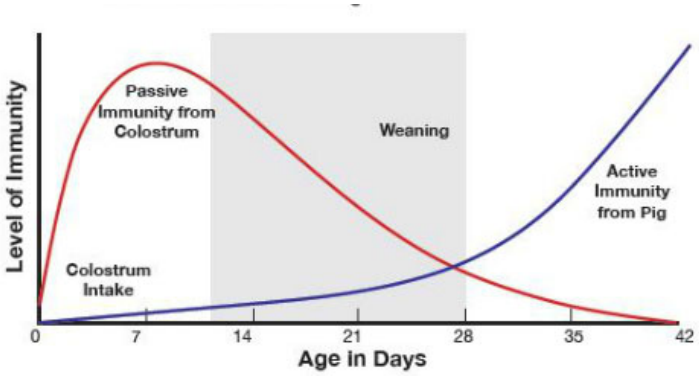

Figure 1. Evolution of passive and active immunity of the piglet (Brown 2017).

As the bases of **active immunity** begin to develop prior to weaning, many authors have focused their efforts on trying to **reinforce its early development** to ensure a rapid establishment in the post-weaning period. For this, it has been proven with the nutritional enrichment of both the mother's diet and the weaned piglets, with substances with immunomodulatory properties.

- **Bioactive compounds from algae**

In this sense, multiple studies have worked with extracts of microalgae or their derivatives to analyze this potential. Thus, V. Van Hamme (2018), (Figure 2), showed that the inclusion of **β-(1,3)–**glucans from algae as **immunomodulatory ingredients,** accelerated the development of the immune response, showing appreciable differences from the first week post-weaning and generating resistance to diseases.

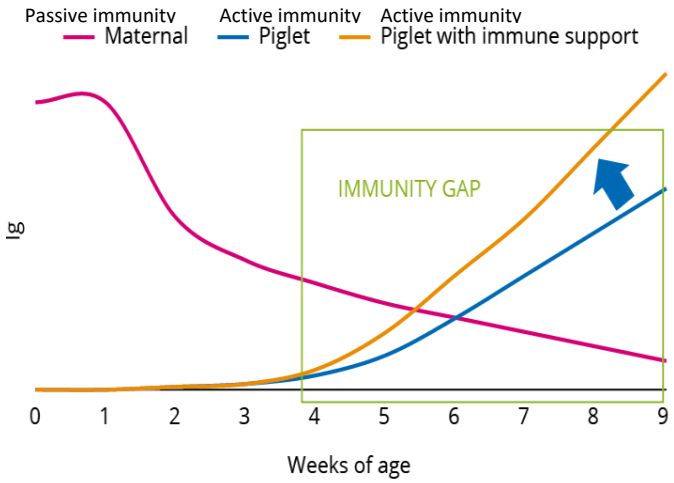

Figure 2. Impact of the inclusion of a β-(1,3)-glucan from algae in the evolution of passive maternal immunity and establishment of the piglet's immune system (Van Hamme 2018).

The results found by Van Hamme (2018) agree on the results of other authors. Thus, Kim et al., (2019), found that the inclusion of a high dose of **β-glucans significantly reduces (*p* <0.05) the frequency of diarrhea** (29.01% vs. 17.28%) in weaned pigs experimentally infected with pathogenic *E. coli* throughout the experimental period (Figure 3). This lower frequency of diarrhea could be explained by a greater immune response, specifically, a reduction in the

permeability of the digestive mucosa *(p* <0.05) and a significant increase in mRNA expression of genes linked to the intestinal barrier **(claudins, occludins and MUC2) in the jejunum mucosa** of pigs exposed to *E. coli.*

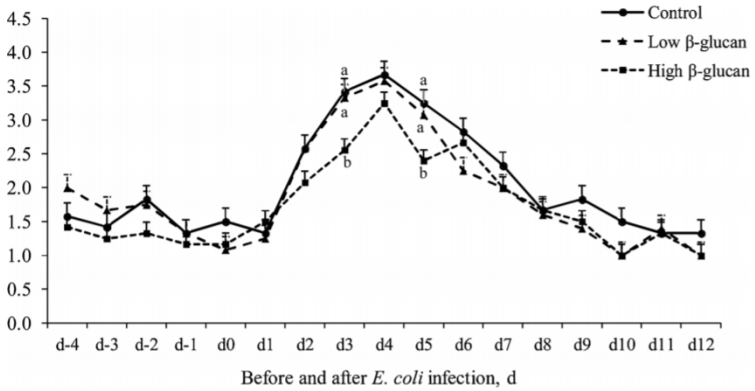

Figure 3: Diarrhea in weaned pigs fed diets supplemented with different doses of β-glucans from algae (Kim et al., 2019).

In this line, Shao et al. (2013) found that the addition of **β-glucans** increased the expression of genes linked to the **synthesis of the proteins involved in the tight junctions** responsible for maintaining cell-cell adhesion, when an immunological challenge occurred in chickens with *Salmonella Typhimurium.* Dietary supplementation with β-glucans can be explained by **different modes of action,** among them, the participation of the **dectin-1 receptor** (Goodridge et al., 2009) and **the response of active immunity linked to T cells**, **modulating the inflammatory response and accelerating the recovery from a bacterial challenge linked to the weaning of piglets** (Kim et al., 2019).

On the other hand, there are other bioactive compounds in microalgae related to their effect on the regulation of the immune response, these compounds are **phytosterols, carotenoids, polyphenols, vitamins and fatty acids** (Caroprese et al., 2014; Valenzuela et., 2015). In fact, Abdel-Daim et al. (2016) related the inclusion of *Spirulina Platensis* with a greater immune response and lower inflammatory profile in animals, which was explained thanks to its high content of functional bioactive compounds, with antioxidant and anti-inflammatory activities, including phenolic phytochemicals (Jensen et al., 2015) and phycobiliprotein C-phycocyanin (Riss et al., 2007).

It has also been observed that the inclusion of **spirulina or immulina** (derivatives of spirulina) produced **an increase in the proliferation of CD4 + T lymphocytes** (Nielsen et al., 2010) and Luescher-Mattli, (2003) found **higher levels of cytotoxic lymphocytes** in animals supplemented with spirulina, relating it to a **higher** rate of establishment of the **innate response, rate of phagocytosis and production of antibodies.** Furthermore, Liang et al. (2004) showed that the inclusion of these microalgae had a positive effect on the **prevalence of *Lactobacillus* in the gut**, increasing the resilience to dysbiosis and decreasing the prevalence of other pathogens.

Microalgae also show important **enhancing effects on metabolic function, linked to the redox potential and the level of lipid oxidation, thanks to their ability to synthesize ω-3 polyunsaturated fatty acids, such as alpha-linolenic acid (ALA), docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA)** (Nayak et al. 2018). In this way, **polyunsaturated fatty acids,**

being one of the fundamental components of cell membranes, **increase the resistance of cell membranes to pathogenic attacks** and **have a modulating effect on inflammation**, which improves the establishment of effective immunity. Furthermore, its effect on membrane stability is especially important in cells with a high multiplication rate, such as enterocytes, hepatocytes, lymphocytes and monocytes (Zhang et al. 2014).

- **Research and development**

On the other hand, in addition to a careful scientific review and extensive experience in animal production, the development of **GreenTonic** has been based on the participation of a **European research project H2020 called Saltgae.** This international participation, with an investment of 9.8 M€ and the participation of 19 partners, has allowed a close collaboration with the algae-producing sector and to value the bioactive compounds of algae as alternatives and nutritional supplements in animal production. The result of the analysis has allowed to obtain a product based on the bioactive components of the algae with better productive and physiological results during the initial development of the piglet, as well as the selection of the most purified sources of these compounds, which have been combined to support and stimulate the early development of the immune system, seeking synergies between bioactive compounds and reducing the proliferation of the main pathogens that affect early stages of the piglet development.

- **Conclusion**

The complex composition of microalgae, rich in bioactive compounds, such as **phytosterols, carotenoids, vitamins, β-glucans, polyphenols and polyunsaturated fatty acids,** has a complex effect on the multiplication and resistance of cells to pathogens. Thus, **their inclusion in the diet of animals and humans has shown a strong reinforcement of the innate response and a significant decrease in bacterial proliferation, demonstrating important immunostimulatory properties.**

In this context, the careful selection of **GreenTonic components** defines it as a **highly palatable hypertonic drink for piglets** from the second day of life, which stimulates its **voluntary consumption by strengthening the digestive system and providing vigor, vitality and immunostimulation thanks, among other components, to the effect of the algae included in its composition.**