A new option for immune stimulation – marine algal polysaccharides

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Today, all poultry production is very concerned with vaccination. This is an essential technique for the protection of flock health which, however, entails significant costs for stock breeders.

Maximising the efficiency and profitability of prophylactic vaccination strategies is therefore of major importance. To achieve this, new avenues are constantly explored.

One of them concerns the use of new molecules extracted from seaweeds to help optimise the stimulation of the natural defences of the body and its response to vaccination strategies.

The body's response to the aggression of a pathogen is based on two types of immunity. They are the innate immune response and the adaptive response (Table 1).

Innate immunity

The innate response is the first line of defence against pathogens. It is activated immediately and acts very quickly. This immune response can be found in all animals. It will be the same whenever the body encounters that pathogen.

However, the body does not retain a memory of the infectious agent. The mechanism of action of this type of immunity consists in recognising the molecular patterns shared by numerous pathogens, which are essentially represented by membrane fractions (glycocalyx).

The various elements that contribute to the innate immune response are the following:

- Physical barrier (mucous membrane, skin, mucus, villi etc).
- Phagocytic cells, such as the macrophages.
- Natural Killer (NK) cells.
- Certain cytokines, which deliver signals warning the body of a danger.
- Complement system.
- Toll-Like Receptors (TLR), a family of membrane receptors only recently discovered. They control the expression of molecules that fight against infectious agents (directly or indirectly, via effector cells, and by recruiting the activation of the adaptive immune system).

The elements associated with the innate immune response can act on the pathogen directly or indirectly, by producing effector cells (cytokines etc).

The latter will subsequently trigger the adaptive immunity by activating the T and B cells.

Adaptive immunity

Unlike the innate response, the acquired or adaptive response occurs in vertebrates only. During the first encounter with a given pathogen (primary infection), it acts as the body's second line of defence. Its activation then takes some time – known as latency.

However, this response system memorises the pathogens it encounters and when the body is again exposed to them the latency is much shorter and the immune system reacts to the aggression almost immediately.

Adaptive immunity is specific: it recognises the molecular patterns of already encountered pathogens.

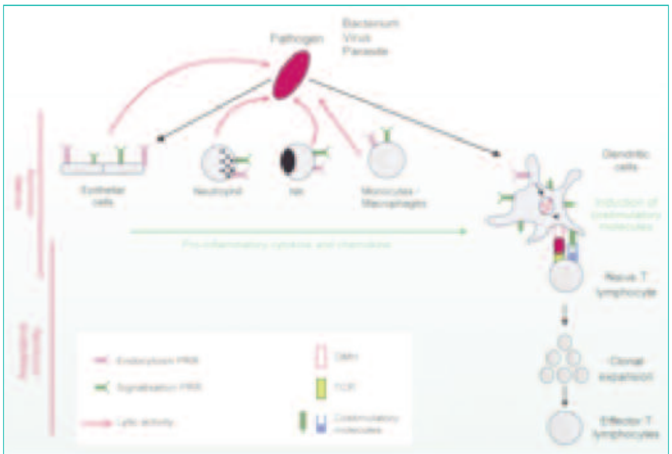
The various elements that contribute to the adaptive immune response are the following:

- T cells.
- B cells.
- Antibodies.
- Ig, TCR, CTL, antibody (AB)-producing plasma cells + coupled aid of the innate immunity effectors (Fig. 1).

	Innate immunity	Adaptive or acquired immunity
Chronology: Primary infection	Quick response: first barrier against pathogens	Second line of defence: latency (about 7 days)
Chronology: Repeated infections	Identical to the primary response	Immune memory => Latency close to zero
Specificity	Non-specific response	Specific response (Ig and TCR)
Recognised molecular patterns	Invariable and common to numerous pathogens	Specific to the infectious agent
Cellular and molecular effectors	Complement, phagocytic cells and certain cytokines	CTL (cytotoxic T cells) and anti-body producing plasma cells, with the help of innate effectors

Table 1. Innate immunity/acquired immunity: two complementary and cooperative systems.

Fig. 1. Dendritic cells provide the coupling between the innate and the adaptive immunity.



Seaweeds: a new source

In recent years more and more publications have brought to the forefront the relevance of seaweeds in numerous biological applications, particularly to immune mechanisms, taking special interest in some of their components, namely the sulphated polysaccharides.

These are complex carbohydrates which do not occur in terrestrial plants. They are supposed to influence the immune system by a vast number of still poorly understood pathways.

Sulphated polysaccharides

Polysaccharides represent a structurally diverse class of macromolecules which are relatively widespread in nature.

There are simple and complex forms of polysaccharides. Unlike proteins and nucleic acids, polysaccharides contain repetitive structural features which are chains of monosaccharide residues joined together by glycosidic bonds.

Thus, they form polymer (-type) structures represented in the form of chains that may be homogenous (homopolysaccharides) or not (heteropolysaccharides).

The simple forms are the homopolysaccharides composed of a single type of sugar, linked in an

Continued on page 29

Continued from page 27
essentially linear manner (starch, glycogen, cellulose). They are essentially structural compounds or mechanisms of energy storage in an easily releasable form.

Their structure may become more complex owing to their capacity of establishing links at various levels of each elementary unit, allowing thus the development of branching structures in three dimensions. These are the branched homopolysaccharides.

Structural variability

The nucleotides in nucleic acids and the amino acids in proteins can interconnect in only one way, while the monosaccharide units in oligosaccharides and polysaccharides can interconnect at several points to form a wide variety of linear or branched structures.

For instance, the number of possible permutations for four different sugar monomers can attain up to 35,560 unique tetrasaccharides, while four amino acids can form only 24 different permutations.

This explains the fact that, among macromolecules, polysaccharides provide the highest capacity for carrying biological information, as they have the greatest potential for structural variability.

In addition, one of the particularities that numerous marine polysaccharides possess is their polyanionic character, which confers them a high chemical reactivity.

Of these anionic polysaccharides, the majority of those which occur macroalgae are sulphated polysaccharides: galactan (agar, carraghenans), ulvans, fucans (Table 2).

The ulvans, for example, the water soluble polysaccharides found in green seaweed of the order Ulvales (Ulva and Enteromorpha), have sulphate, rhamnose, xylose and iduronic and glucuronic acids as their main constituents.

Ulvan structure shows great complexity and variability as evidenced by the numerous oligosaccharide repeating structural patterns identified.

The main repeating disaccharide units reported are of ulvanobiouronic acid 3-sulphate type, containing either glucuronic or iduronic acid. In addition, a few repeating patterns can be found that contain sulphated xylose replacing uronic acid or glucuronic acid on the O-2 binding/link of the rhamnose-3-sulphate units.

This huge variability in the polysaccharide structure provides the flexibility required for exact regulatory mechanisms in different cell-cell interactions in higher organisms.

Sulphation in particular seems to be conducive to various biological activities noted in polysaccharides extracted from marine macroalgae.

Effect on immunity

Sulphated polysaccharides, which are widespread in macroalgae, have been shown to possess anti-infectious (anti-viral, anti-bacterial, anti-tumoral), antioxidant and anti-thrombotic activities, as well as immune-modulating activities that might find relevance in stimulating the immune response or in controlling the activity of immune cells in order to mitigate negative effects such as inflammation (Fig. 2).

One of the pathways of marine sulphated polysaccharides, which has been emphasised recently, is their role in the activation of TLR (Toll-Like Receptor).

Indeed, more and more studies are demonstrating that marine algal polysaccharides can influence the innate immune response by binding to recognition receptors called PRR (Pattern Recognition Receptors), such as the mannose receptors or Toll-like receptors (TLR) of phagocytic cells, including and especially macrophages.

Toll-like receptors (TLRs) are transmembrane proteins which detect invading pathogens by binding to ancestral molecules of microbial origin called Pathogen-Associated Molecular Patterns (PAMPs).

The PAMPs contact at TLR level triggers a cascade of responses resulting in the expression of inflammatory response genes.

In poultry, these recently identified receptors have been numbered from 1 to 10 (TLR1-TLR10).

On contact with their respective PAMPs, TLR specifically activate a signalling pathway leading to the activation of NF-kB (Nuclear Factor-kappa B) and AP1 (Activator Protein 1) transcription factors regulating the expression of inflammatory cytokines such as TNFα, IL-1 or IL-6.

It therefore now appears that TLR play a key role in the adaptive immune response, but the signals produced by their activation lead to the activation of numerous other cells and functions of the immune system, which makes them essential elements of both the innate immune mechanisms and of adaptive immunity.

The activity of some sulphated algal polysaccharides as TLR activating agents might be the result of a certain structural similarity between these marine polysaccharides and bacterial lipopolysaccharides (LPS).

Bacterial LPSs are a type of structure occurring at the surface of their external membrane and recognised as bacteria-specific recognition elements.

Table 2. Classification of marine sulphated polysaccharides (MSP).

Seaweed		
Brown	**Red**	**Green**
Alginate, fucoïdan	Galactan, carrageenan	Ulvan
Fucose, xylose, uronic acid, galactose sulphate	Sulphated agalactose, 3,6-Anhydrogalactose	Sulphated rhamnose, sulphated aldobiuronic acid
	Kappa, iota, lambda types	

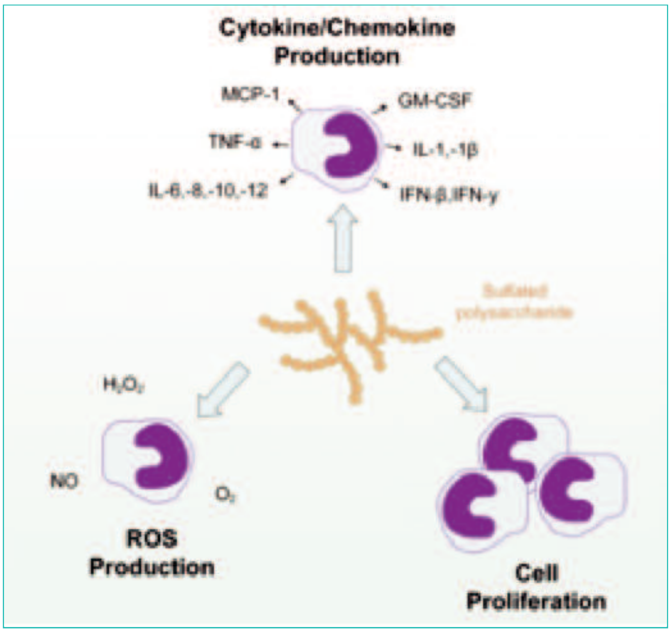


Fig. 2. Illustration of the immunomodulating activities of sulphated polysaccharides by means of macrophage activation.

Possible applications

In conclusion, seaweeds appear to contain sugars in the form of polysaccharides, some of which – are sulphated polysaccharides – are complex polyanionic structures which possess various biological properties.

A vast number of studies have already evidenced the effects of some of these sulphated polysaccharides, particularly the fucoidans, the carraghenans and the ulvans, on certain mechanisms of inflammatory response and on immunity.

The identification and selection of these polysaccharides extracted from suitable macroalgae makes it possible to envisage the use of these molecules as agents for the stimulation of the various mechanisms associated with the body defence and, in particular, of the innate immunity mechanisms.

Within the framework of the potential applications in the fields of animal breeding and animal health two non-exclusive strategies can be proposed:

- Regular sequential intakes for a general stimulation of the body's state of defence: With a regular intake not connected with vaccination, they allow the strengthening of the body's state of defence. Repeated use allows the development of a 'basic' immune system and the boosting of the state of defence of the innate system.

The use of polysaccharides upstream or downstream of a prophylactic program may be an asset in enhancing the level of immune protection of an individual or group of individuals within a flock and in contributing to a better control of the infectious pressure on the flock, preventing the appearance of recurrent infectious pathologies.

- Targeted intakes within the framework of a vaccination program: As part of a vaccination program, they would enhance the vaccine protection. As potential 'adjuvants' in future vaccination strategies for poultry, this would definitely provide the possibility to improve the intake and persistence of the vaccine and thereby to improve the technical and economic performance of vaccine prophylactic programs. ■

References are available from contact@amadeite.com