Immunomodulators as efficient alternatives to in-feed antimicrobials in pig production?

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ABSTRACT
In animal production, alternative strategies to in-feed growth-promoting antibiotics are being developed to increase the resistance of piglets to disease, especially during the weaning transition where they are highly sensitive to digestive disorders. The incorporation in feed of substances able to modulate immune functions, and thus to stimulate host defence, is a strategy which has gained increasing interest in animal research in past decade. This review will focus on main components known to have immunomodulatory properties, and which have been the subject of in vivo nutritional investigations in pig: yeast derivates, different plant extracts and animal by-products. Yeast derivates (β-glucans and mannans) are known to interact with immune cells, particularly phagocytic cells. However, inconsistent results have been observed when they have been fed to piglets, which questions their ability to target through the oral route the sensitive immune cells. The literature dealing with effects of different plant extracts on pig immunity offers some promising results, but is still too scarce and disparate to ascertain positive effects. To date, the most promising alternative is probably represented by spray-dried animal plasma, whose positive effects on piglet immunity and health would be mainly provided by specific antibodies, but also through non-specific competition of some plasma components with bacteria for intestinal receptors.

Keywords: pig, immunity, disease sensitivity, feed additive, immunomodulators

INTRODUCTION
To improve both performances and health status of individuals, adding sub-therapeutic doses of antibiotics to feed has been widely used in the pig industry over the past decades (Cromwell, 2002). However, their use as growth-promoters has been completely banned throughout Europe since 01 January 2006 (Regulation (EC) No 1831/2003), and alternative strategies are now needed. In pig, the weaning is a very stressful event, and the post-weaning

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period is characterised by an immediate, but transient, drop in feed intake with alteration in gut architecture and function, making piglets highly sensitive to digestive diseases (Lallès et al., 2004). Various nutritional approaches have been proposed to help piglets to cope with this transition (Lallès et al., 2007), including the supplementation of the diet with substances that increase appetite or have anti-microbial and/or immune-stimulating properties. Strategies aiming at boosting natural host defences (e.g. immune-modulators) are currently attracting a greater level of attention. Indeed, the correct functional development of the gastro-intestinal tract is of crucial importance in controlling potential pathogens during the neonatal and post-weaning period. Weaning affects the ontogeny of immune functions, largely as a consequence of the withdrawal of milk, and its important implication for passively modulating immune responses. The aim of immune-modulating substances is to favour “appropriate” active responses from both innate and acquired immunity in piglets. In a perspective of short or mid-term application in pig farm, a balance-sheet of the potential use of immunomodulators in pig nutrition is needed. For this purpose, this paper will focus on what is currently published concerning substances that may enhance immune function and health, and whose properties have been investigated in vivo through in feed supplementation in pig. Specifically, the use of yeast derivates, plant extracts and animal by-products will be discussed.

YEAST DERIVATES

A variety of polysaccharides from different natural sources would be able to modulate immune functions. β-D-glucans and the carbohydrate portion of mannoproteins, the α-D-mannans, belong to this category through specific interactions with different immunocompetent cells, such as macrophages and polymorphonuclear cells (Tzianabos, 2000). Those two components are found in large quantity in yeast cell wall, which represents the main source for in-feed additives currently used in animal production.

Glucans

Different in-feed complements containing β-glucans are commercialized in animal industry, and several preparations have been tested for their effects on pig immunity. One main problem is that the source, the composition and the purity of these products often remain unknown, which could explain the high variability of the incorporation rate, as well as the discrepancies between studies.

In pig, β-glucans have been shown to have anti-inflammatory properties (Table 1). They can prevent the elevation in pro-inflammatory cytokines whilst enhancing the production of anti-inflammatory cytokines in response to a challenge with lipopolysaccharide (LPS) (Li et al., 2005; Li et al., 2006).
Glucans can modulate the acute phase response, whose regulation is known to be orchestrated by pro-inflammatory cytokines like IL-1, IL-6 or Tumor Necrosis Factor-α (TNF-α) (Baumann and Gauldie, 1994). They partly suppress the increase in blood haptoglobin concentration that occurs during the two weeks following an early weaning (Dritz et al., 1995). At the intestinal level, anti-inflammatory properties of glucans are more difficult to assess as they have been shown to increase simultaneously the pro-inflammatory (TNF-α, IL-1β) and the anti-inflammatory (IL-1 receptor antagonist) responses in pigs challenged 4h earlier with LPS (Eicher et al., 2006).

As a known target for glucans, the function of neutrophils and macrophages has been also investigated (Brown and Gordon, 2003). However, dietary β-glucans have no consistent effects on neutrophil phagocytic activity (Dritz et al., 1995; Sauerwein et al., 2007). Similarly, the ability of peripheral blood lymphocytes to proliferate after a stimulation with mitogens in vitro is not modulated by dietary glucans (Hiss and Sauerwein, 2003). However, the production of the different classes of Igs would be influenced in a dose-dependent manner by glucans, lower dose favouring IgA and higher dose depressing IgG responses (Sauerwein et al., 2007).

Specific response to a systemic immunisation has produced contrasting results concerning the effects of dietary supplementation with β-glucans, which was either lowered for atrophic rhinitis vaccine (Hahn et al., 2006), or enhanced in response to an immunisation with ovalbumin (Li et al., 2005). β-glucans had however no effects on the efficiency of a vaccination with porcine reproductive and respiratory syndrome (PRRS) virus (Hiss and Sauerwein, 2003).

Contrasted effects of glucans on immunity are consistent with their unequal ability to promote growth and health. Mostly without any growth-promoting properties (Dritz et al., 1995; Hahn et al., 2006; Sauerwein et al., 2007), glucans can in some situations stimulate growth and/or feed intake (Decuypere et al., 1998; Li et al., 2006), but also depress performances (Dritz et al., 1995; Kim et al., 2000). In the only study to our knowledge that refers to an infectious challenge (with Streptococcus suis), the sensitivity of piglets fed with glucans was highly compromised with a mortality rate reaching 50% (Dritz et al., 1995).

**Mannans**

The ability of mannans to “adsorb” enteric pathogens and to modulate immune functions would be responsible for their potential protective activities (Sohn et al., 2000). However, the influence of dietary mannans on gut health and immune function in swine is not well documented (Table 1). At the intestinal level, macrophage phagocytosis seems to be enhanced in lamina propria by the inclusion of mannans in diet (Davis et al., 2004a). It has also been reported that the recruitment of lymphocytes into the small intestinal lamina propria was reduced in piglets fed mannans (Lizardo et al., 2008), and
that their subset was influenced: a lower ratio of \(\text{CD}^3\text{CD}^4^-/\text{CD}^3\text{CD}^8^+\) T cells after a 3-week supplementation with mannans (Davis et al., 2004a).

Systemic immune responses to dietary mannans have been more widely studied. Under normal breeding conditions, the blood concentration in \(\alpha\)-1-acid glycoprotein, a protein of the acute phase response, is insensitive to a mannan supplementation (Davis et al., 2004a). However, in response to a supplementation with phosphorylated mannans, a decreased blood neutrophil:lymphocyte ratio has been observed (Davis et al., 2004a). This increased lymphocyte population among leukocytes could be linked more to B than to T cells. Indeed, 3% of brewer’s yeast (which corresponds to a final level of 0.16% of mannan oligosaccharide) tended to increase the piglet serum level of IgG when used alone, and substantially increased this level when fed with citric acid (White et al., 2002). Conversely, blood proportions of CD4^+ or CD8^+ lymphocytes are insensitive to mannan supplementation (Kim et al., 2000). The ability of peripheral blood lymphocytes to proliferate in vitro is mostly not affected by dietary mannans (Davis et al., 2002; Davis et al., 2004a; Davis et al., 2004b), but can also be depressed in some conditions (Davis et al., 2004b).

In piglets challenged with Salmonella enterica serotype Typhimurium, serum haptoglobin concentrations were increased in mannan fed-piglets 6 and 13 days post-infection as compared to piglets fed the basal diet (Burkey et al., 2004). Contrary to carbadox, mannans failed to reduce the length of the period of hyperthermia observed after infection with S. enterica and did not promote growth (Burkey et al., 2004). Accordingly, in piglets challenged or not with an enterotoxigenic strain of E. coli K88, mannans did not consistently reduce the intestinal colonization or fecal excretion of ETEC (White et al., 2002).

As for glucans, the influence of mannans on immunity is not always reliable, as well as their effects on piglet performances and health.

**PLANT EXTRACTS**

Plant extracts have gained increasing interest as possible feed additives for animal productions (Windisch et al., 2008). However, plants and their bioactive components, when known, are very diverse and their potential to enhance pig health and immunity has only been scarcely evaluated in vivo (Table 1).

**Herbaceous plants**

Mixtures of essential oils based on thymol and carvacrol, whose major sources are thyme and oregano respectively (Burt, 2004), seem promising due to their potential immunomodulatory properties (Woollard et al., 2007). An extract of Origanum vulgare, enriched with thymol and carvacrol in similar proportions, was reported to protect low-weight growing-finishing pigs from disease (Walter and Bilkei, 2004). This health benefit was associated with an increased proportion of CD4^+, CD8^+ and double positive T cells in peripheral blood and mesenteric lymph nodes (Walter and Bilkei, 2004). Thymol used
alone enhances total IgA and IgM serum levels and exhibits some local anti-inflammatory properties, as indicated by a reduction in TNF-α mRNA in the stomach of post-weaned pigs (Trevisi et al., 2007). However, a plant extract containing 6% of carvacrol and 0.14% of thymol, incorporated at 0.05 to 0.15% in pig diet, had no effect on the plasma levels of acute phase proteins (Muhl and Liebert, 2007), and the inclusion of a commercial plant product composed of oregano oil mixed with anis and citrus oils did not improve health status of piglets (Kommera et al., 2006). In vitro, cinnamaldehyde, the main component of cinnamon essence, also has immunomodulatory properties (Koh et al., 1998). A plant extract containing 5% of carvacrol (Origanum spp.), 3% of cinnamaldehyde (Cinnamomum spp.) and 2% of capsicum oleoresin (Capsicum annum), included in the feed at a 0.03% level, led to a decreased number of jejunal intra-epithelial lymphocytes, and an increased number of lymphocytes in the colonic lamina propria (Manzanilla et al., 2006). Conversely, mononuclear cell subsets from ileal Peyer’s patches were not affected by this plant extract combination and only the percentage of B lymphocytes was reduced in lymph nodes of piglets (Nofrarias et al., 2006). Those immune modulations had however no effects on performances and health.

Plants of the Echinacea family are an indicator of “good health” of pastures. The main bioactive components of Echinacea purpurea are chicory acid and alkamids. When included as juice or cobs in the post-weaning diet, growth performances are not improved, but feed efficiency tends to be increased (Maass et al., 2005). However, blood parameters, including cell count and lymphocyte proliferation, were not modified by dietary treatment in this study, but this could be attributed to the good health status of piglets throughout the trial. The response to immunization of piglets to a vaccine against Erysipelothrix rhusiopathiae was enhanced by the inclusion of E. purpurea into the diet of finishing pigs (Maass et al., 2005). Further studies would be required to confirm these results in post-weaning period.

Whilst the bulk of β-glucans used in feed industry is derived from yeast cell wall (see previous section), properties of β-glucans from the Chinese herb Astragalus membranaceus have also been investigated. Yuan et al. (2006) reported that dietary A. membranaceus increases the white blood cell count, mainly through the contribution of CD4+ lymphocytes. The proliferation of T cells isolated from peripheral blood in weanling pigs was also increased in a dose-dependent manner in β-glucans-fed piglets (Mao et al., 2005). Concomitantly, β-glucans from Astragalus increased blood concentration in IL-2 and interferon-γ (IFN-γ), whereas IL-4 and IL-10 concentrations remained unchanged (Mao et al., 2005; Yuan et al., 2006). This cytokine profile suggests a Th1 bias, and thus an enhancement of cellular immunity. Conversely, plant β-glucans do not seem to influence humoral immunity, as indicated by the specific antibody titres following immunisation with ovalbumin (Yuan et al., 2006). Moreover, when supplied at moderate doses, glucans from A. membranaceus
can counteract the increased plasma concentrations of IL-1β and prostaglandin E2 induced by a LPS challenge (Mao et al., 2005). These immune modulations conferred by vegetal glucans (anti-inflammatory properties, increased T-lymphocyte proliferation) may be beneficial for the piglets to fight against infections, but this need to be specifically demonstrated.

Genistein and daidzein, two isoflavones found in soybean products, were also suggested to act as immune-modulators when given orally. After oronasal infection of piglets with PRRS virus, dietary daidzein failed to decrease serum titres of virus (Greiner et al., 2001b), whereas genistein minimised the viremia from day 4 to day 24 post-inoculation, as well as the serum concentration of IFN-γ (Greiner et al., 2001a). Serum α-1-acid glycoprotein concentration was not modulated by daidzein (Greiner et al., 2001b), but was increased during periods of high viremia by genistein (Greiner et al., 2001a). This enhanced α-1-acid glycoprotein response in genistein-fed piglets supports the hypothesis of a greater and more effective immune response, which could explain the lower viremia (Greiner et al., 2001a). Accordingly, lower serum IFN-γ concentration in genistein-fed animals is in agreement with the greater virus elimination and a quicker return of IFN-γ to basal levels (Greiner et al., 2001a). Despite the different effects on viremia, both isoflavones were efficient in promoting growth in piglets challenged with PRRS virus, which suggests that their mechanisms of action would differ. It would be of interest in future to test their ability to enhance immune responses and health following a challenge with an intestinal pathogen.

Ligneous plants

Saponins that are widely used as a vaccine adjuvant are contained in the South American tree Quillaja saponaria (Kensil et al., 2004). However, dietary treatment of piglets with crude soap bark of Q. saponaria did not counteract the negative effects on feed intake and growth induced transiently by a challenge with Salmonella enterica Serovar typhimurium (Turner et al., 2002b). Q. saponaria also failed to modulate the rise in serum haptoglobin, α-1-acid glycoprotein and IgM concentrations induced by this challenge 7 and 14 days post-infection (Turner et al., 2002b). The phagocytic function of peripheral white blood cells tended to be depressed in challenged pigs but only when fed with high doses of Q. saponaria (Turner et al., 2002b). It has been suggested that these “weak immune modulations” may be due to the low purity of the extract used (Ilsley et al., 2005), i.e. a low content in saponins and a high content in tannins known to have anti-nutritional properties (Singh et al., 2003). Thus, Ilsley et al. (2005) incorporated a purified saponin extract from Q. saponaria in the diet, alone or in combination with curcumin which has been shown to modulate lymphocyte mediated immune functions in mice (Churchill et al., 2000). Whereas piglet immune responses were not influenced by curcumin, the feed intake and serum IgA, IgG and C-reactive protein
concentrations were transiently increased in saponin-fed piglets (Ilsley et al., 2005). The subsequent negative impact of saponin on feed utilization could result from increased dietary requirements to mount an immune response (Ilsley et al., 2005). However, the impact on health of such an increased immune response still needs to be demonstrated.

**Seaweed extracts**

Seaweeds extracts are also known to modulate immune functions (Yoshizawa et al., 1993). In pig, only one study presents data on influence of a seaweed extract on immune functions. An extract derived from Ascophyllum nodosum incorporated at different levels was tested in piglets orally challenged with Salmonella enterica Serovar typhimurium (Turner et al., 2002a). Increasing levels of A. nodosum extract tended to linearly enhance the feed intake, but decreased the feed efficiency during the 4 weeks following the weaning of piglets. Dietary A. nodosum had no influence on immune responses whether the piglets were infected or not, but the challenge with Salmonella had only moderate effects on piglets. This suggests that A. nodosum extract probably has only little direct effect on gut-associated lymphoid tissue in pig, but further studies would be required to confirm this finding.

**ANIMAL BY-PRODUCTS**

**Spray-dried animal plasma**

Spray-dried plasma (SDP) is a by-product of commercial bovine or porcine slaughtering facilities, whose effects on local intestinal immune responses have been fairly well studied in pigs. Results are concordant and reveal that SDP prevents the infiltration of gut associated lymphoid tissue by macrophages and lymphocytes (Jiang et al., 2000; Nofrarias et al., 2006; Nofrarias et al., 2007). This decreased infiltration is likely to be a reflection of a lower antigenic stimulation of gut associated lymphoid tissue (Nofrarias et al., 2007). Additionally the jejunal expression of the pro-inflammatory cytokines IL-8 and TNF-α has been shown to be decreased in SDP-fed piglets challenged with ETEC K88 (Bosi et al., 2004). This decreased local inflammatory response may be explained by a lower colonization or binding of E. coli to intestinal receptors as assessed by their lower serum IgA titre and histopathological score (Bosi et al., 2004).

Concerning systemic immune responses, dietary porcine plasma did not modulate the increase in blood white blood cell count that normally occurs during the 2 weeks following the weaning (Jiang et al., 2000; Nofrarias et al., 2007). SDP did not influence serum IFN-γ or TNF-α under basal conditions (Touchette et al., 2002), but when stimulated with an intraperitoneal LPS injection, SDP-fed piglets showed increased serum levels of IFN-γ and TNF-α associated with severe intestinal damage, suggesting that SDP-fed piglets would
be more susceptible to some immunological challenges (Touchette et al., 2002). Similarly, in piglets intravenously challenged with LPS, SDP increased the serum level of IL-6 and IL-1β, but SPD did not modulate mRNA cytokine levels in liver, thymus or spleen (Frank et al., 2003). Conversely, SDP supplementation led to a decrease in C-reactive protein concentration but not in haptoglobin (Frank et al., 2003). However, the immune stimulus used (intraperitoneally or intravenously injection of extremely high level of LPS, 75-150 µg /kg of BW) might not be representative of natural pathogen exposure via the gastrointestinal tract.

The growth-promoting properties of SDP that are usually reported (Jiang et al., 2000; Bosi et al., 2004; Nofrarias et al., 2006; Niewold et al., 2007; Nofrarias et al., 2007) are more commonly observed with SDP from porcine than bovine origin (van Dijk et al., 2001). Moreover, their antimicrobial/immunomodulatory properties seems more beneficial when piglet growth is compromised, such as in conventional on-farm nursery setting as compared to “cleaner” off-site nursery (Coffey and Cromwell, 1995), or in pigs early-weaned (Torrallardona et al., 2002). This could be related to health promoting properties of SDP observed in challenged piglets. Indeed, in piglets challenged via oral route with an ETEC strain, inclusion of porcine SDP in diet can reduce post-weaning diarrhoea and increase growth of piglets that are positive for F4 receptor (Niewold et al., 2007). This effect is enhanced in piglets fed a diet containing a SDP obtained from pigs previously immunised with a vaccine against neonatal E. coli, where a concomitant decreased ETEC excretion can be observed. These results confirm that protection conferred by SDP would be mainly due to specific antibodies, but also highlight the implication of less specific components. Indeed, the use of a plasma powder depleted of antibodies directed towards adhesion factors of the challenge strain of E. coli (O141:K85ab expressing the F18ac fimbriae) was efficient in protecting piglets against oedema disease (Nollet et al., 1999). Moreover, in piglets orally challenged with different strains of pathogenic E. coli (van Dijk et al., 2002; Bosi et al., 2004; Yi et al., 2005; Torrallardona et al., 2007), SDP generally prevents growth retardation and clinical signs, a phenomenon which is however inconstantly associated to decreased circulation or excretion of pathogenic strains. This supports the theory of a direct competition of SDP with intestinal receptors for pathogenic E. coli, more than a direct antimicrobial effect (Bosi et al., 2004). Specific and unspecific mechanisms of action of SDP are likely, and may act synergistically to decrease disease susceptibility of piglets.

**Other animal by-products**

Bovine colostrum has a critical role in postnatal health, through the passive transfer of antibodies and of growth- and anti-microbial factors (IGF, epidermal growth factor, lactoferrin, etc) (Pakkanen and Aalto, 1997). In piglets, bovine colostrum has been shown to enhance mucosa restoration by stimulating
migration of epithelial cells along the crypt-villous axis in intestine, and by
decreasing apical cell apoptosis (Huguet et al., 2007). Recent studies would
suggest that bovine colostrum has immunomodulatory properties that are
directly related to specific region of the porcine gut-associated lymphoid tissue.
Dependent upon the region studied, bovine colostrum could lead to Th1 (IL-2,
IFN-γ, IL-12) or Th2 (IL-4, IL-10) cytokine profiles, a bipolarity activity that
would be of importance in a context of exposure to a wide range of antigens
(Boudry et al., 2007). In other respects, bovine colostrum significantly
decreased the total number of mononuclear cells in ileal Peyer’s patches, but
their proliferative responses were increased (Boudry et al., 2007). Accordingly,
the ability of lymphocytes to proliferate after stimulation with different
mitogens was also increased in other compartments like mesenteric lymph
nodes, spleen or blood. To our knowledge, health benefits of bovine colostrum
that could result from the orchestration of immune responses have not yet been
studied and could be the subject of further investigations.

The use of lactoferrin is also promising, as it seems efficient in preventing
diarrhoea in piglets (Shan et al., 2007; Wang et al., 2007). Furthermore,
lactoferrin has been shown to modulate some systemic functions of cellular
immunity (increased ability of peripheral blood and spleen lymphocytes to
proliferate) and humoral immunity (increased serum concentrations in IgA, IgM
and IgG) in pig (Shan et al., 2007). To our knowledge, its impact on local
intestinal immunity as well as its potential to enhance specific immune
responses has not yet been investigated in pig.

**CONCLUSION**

Literature dealing with natural alternatives to in-feed antibiotics and their
impact on pig immunity is scarce. To our opinion, two main raisons are
responsible for this lack of knowledge. First, the total ban of in-feed antibiotics
is recent and secondly, in-feed alternatives that can act directly on immunity,
and not through the control of microflora, have only recently gained interest.
Thus published works on that topic may arise in the following years.

Immunomodulatory effects of several substances are often first described *in vitro*,
and this review highlights that most of them are less potent when tested *in vivo*
as feed additives, or not consistent between studies. One main problem is
that, in many cases, the level of the feed additive in the final diet is not
available. Thus, conflicting results obtained from an *a priori* similar additive
may be attributed to factors such as the feed content of the bioactive component
tested. Inconstant or conflicting results may also arise from variations in the
origin, structure or purity level of the added compound. Moreover, when
administered through the oral route, additives will be submitted to a myriad of
events (feed processing and storage, interactions with other nutrients, digestive
processes…) which may limit their activities before reaching the cells of the gut
associated immune system. In spite of those experimental design considerations,
the positive or negative impact on health of immune modulations engendered by feed additives is difficult to assess. The strategy underlying immunomodulations is to identify aspects of the host response that will enhance or complement a “desired immune response”, to allow the host to better fight against invading micro-organisms during the course of infection. The main problem is to define what is considered as a “desired” immune response. Immune responses, and their consequences, are more easily understood when health status of piglets is objectively studied. This implies that piglets are not kept under very clean conditions, but are exposed to normal commercial rearing conditions or submitted to immune challenges to establish connections between immune modulations and health.

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challenged with a pathogenic *Escherichia coli*. *Veterinary Microbiology*, 84:207-218.


Table 1. Effects of in-feed immunomodulators tested in post-weaned pig on various measures of performances, immune functions and health

<table>
<thead>
<tr>
<th>Yeast derivates</th>
<th>Weaning age (d)</th>
<th>Supplementation (wk)</th>
<th>Experimental conditions</th>
<th>Growth</th>
<th>Feed Intake</th>
<th>G:F ratio</th>
<th>Immune traits</th>
<th>Health status</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucans&lt;sup&gt;A&lt;/sup&gt;</td>
<td>14</td>
<td>4</td>
<td>S</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>n.d.</td>
<td>n.d.</td>
<td>Dritz et al., 1995</td>
</tr>
<tr>
<td>Glucans&lt;sup&gt;A&lt;/sup&gt;</td>
<td>21</td>
<td>4</td>
<td>S</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>n.d.</td>
<td>Dritz et al., 1995</td>
</tr>
<tr>
<td>Glucans&lt;sup&gt;A&lt;/sup&gt;</td>
<td>21</td>
<td>4</td>
<td>S</td>
<td>—</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Kim et al., 2000</td>
</tr>
<tr>
<td>Glucans&lt;sup&gt;A&lt;/sup&gt;</td>
<td>26</td>
<td>4</td>
<td>S</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Decuypere et al., 1998</td>
</tr>
<tr>
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<td>27-30</td>
<td>4</td>
<td>S</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Blood: ↑IgA, ↓IgG</td>
<td>0</td>
<td>Sauerwein et al., 2007</td>
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<td>Glucans&lt;sup&gt;C&lt;/sup&gt;</td>
<td>28</td>
<td>5</td>
<td>Š</td>
<td>Š</td>
<td>0</td>
<td>0</td>
<td>n.d.</td>
<td>0</td>
<td>Li et al., 2006</td>
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<tr>
<td>Glucans&lt;sup&gt;D&lt;/sup&gt;</td>
<td>14</td>
<td>2</td>
<td>Ch</td>
<td>Š</td>
<td>n.d.</td>
<td>n.d.</td>
<td>Blood: ↓granulocyte and lymphocyte counts Spleen: ↑TNF-α and IL-1β Ra mRNA</td>
<td>n.d.</td>
<td>Eicher et al., 2006</td>
</tr>
<tr>
<td>Glucans&lt;sup&gt;A&lt;/sup&gt;</td>
<td>18</td>
<td>6</td>
<td>Ch</td>
<td>—</td>
<td>n.d.</td>
<td>n.d.</td>
<td>Blood: ↓haptoglobin</td>
<td>Increase sensitivity to S. suis infection</td>
<td>Dritz et al., 1995</td>
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<tr>
<td>Glucans&lt;sup&gt;C&lt;/sup&gt;</td>
<td>28</td>
<td>4</td>
<td>Ch</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>Plasma: ↓IL-6 and TNF-α, ↑IL-10, ↑IGF-1</td>
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<td>Li et al., 2006</td>
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<td>Glucans&lt;sup&gt;E&lt;/sup&gt;</td>
<td>n.i.</td>
<td>8</td>
<td>Vac</td>
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<td>0</td>
<td>0</td>
<td>Blood: ↓specific Ab titer, ↑CD4+ and CD8+ lymphocytes</td>
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<td>Hahn et al., 2006</td>
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<td>4</td>
<td>Vac</td>
<td>0</td>
<td>Š</td>
<td>0</td>
<td>0</td>
<td>n.d.</td>
<td>Hiss and Sauerwein, 2003</td>
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<td>28</td>
<td>4</td>
<td>Ch + Vac</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>Blood: ↑specific Ab titer, ↓IL-6, ↓TNF-α, ↑IL-10</td>
<td>n.d.</td>
<td>Li et al., 2005</td>
</tr>
<tr>
<td>Mannans&lt;sup&gt;F&lt;/sup&gt;</td>
<td>18</td>
<td>5</td>
<td>Š</td>
<td>Š</td>
<td>Š</td>
<td>Š</td>
<td>0</td>
<td>n.d.</td>
<td>Davis et al., 2002</td>
</tr>
<tr>
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<td>19</td>
<td>4.5</td>
<td>Š</td>
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<td>0</td>
<td>Š</td>
<td>Lamina propria: ↑macrophage phagocytosis</td>
<td>n.d.</td>
<td>Davis et al., 2004 (a,b)</td>
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<td>Component</td>
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<td>Concentration</td>
<td>Media</td>
<td>Location</td>
<td>Effect</td>
<td>n.d.</td>
<td>Reference</td>
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<tr>
<td>Mannans(^{F})</td>
<td>21</td>
<td>4</td>
<td>S</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Kim et al., 2000</td>
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<td>Mannans(^{G})</td>
<td>22</td>
<td>4</td>
<td>S</td>
<td>–</td>
<td>–</td>
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<td>Blood: ↑ IgG</td>
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<td>S</td>
<td>Œ</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>Intestine: ↓ IEL</td>
</tr>
<tr>
<td>Mannans(^{G})</td>
<td>11</td>
<td>7,5</td>
<td>Ch</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Blood: ↑ haptoglobin</td>
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**Plant extracts***

<table>
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<th>Media</th>
<th>Location</th>
<th>Effect</th>
<th>n.d.</th>
<th>Reference</th>
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<tr>
<td>Thymol</td>
<td>24</td>
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<td>–</td>
<td>Œ</td>
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<tr>
<td>Glucans</td>
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<td>4</td>
<td>Ch</td>
<td>Œ</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Glucans</td>
<td>28</td>
<td>3</td>
<td>Vac</td>
<td>Œ</td>
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<tr>
<td>Daidzein</td>
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<td>Ch</td>
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<td>Genistein</td>
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<td>Quillaja</td>
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<td>S</td>
<td>0</td>
<td>Œ</td>
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<tr>
<td>Quillaja</td>
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<td>Ch</td>
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<td>Ascophyllum</td>
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<td>Ch</td>
<td>0</td>
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**Animal by-products**

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<td>SDP</td>
<td>14</td>
<td>2</td>
<td>S</td>
<td>Œ</td>
<td>Œ</td>
<td>Œ</td>
<td>0</td>
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<td>SDP(^{i})</td>
<td>20</td>
<td>3</td>
<td>S</td>
<td>0</td>
<td>0</td>
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<tr>
<td>SDP</td>
<td>20</td>
<td>3</td>
<td>S</td>
<td>0</td>
<td>n.d.</td>
<td>Jejunum, colon: ▼ IEL</td>
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<tr>
<td>SDP</td>
<td>14</td>
<td>1</td>
<td>Ch</td>
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<td>n.d.</td>
<td>Spleen, thymus: ▼ IL-1β, IL-6, TNF-α mRNA</td>
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<tr>
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<td></td>
<td></td>
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<td>Blood: ▲ IFN-γ, ▲ TNF-α</td>
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</tr>
<tr>
<td>SDP</td>
<td>17</td>
<td>2</td>
<td>Ch</td>
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<td>Blood: ▼ C-reactive protein, ▲ cortisol, ▲ IL-1β, ▲ IL-6</td>
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<tr>
<td>SDP</td>
<td>17</td>
<td>1.5</td>
<td>Ch</td>
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<td>Prevent jejunal lesions (ulcer, oedema...) to ETEC</td>
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<tr>
<td>Bovine colostrum</td>
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<td>3</td>
<td>S</td>
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<td>Lactoferrin</td>
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<td>0</td>
<td>Prevent diarrhoea</td>
<td></td>
</tr>
</tbody>
</table>

In-feed immunomodulators with a same letter are from a common source; * either refers to the source or the suspected bioactive component; SDP: spray-dried animal plasma; S: standard conditions, Ch: challenge (inflammatory or infectious), Vac: vaccination; n.d.: not determined; n.i.: not indicated; 0: no effect observed; ▼ and ▲ are positive and negative effects respectively observed on performance parameters; iPP: ileal Peyer’s patch; MLN: mesenteric lymph node; WBC: white blood cell; IEL: intra-epithelial lymphocytes.

**Foreword:** some caveats to this summary table must be mentioned: 1/ experimental diets might have contained added antibiotics, 2/ effects of treatment might have been observed in only one replicate or during only a specific period or for only one dose or for a specific environmental infection pressure, 3/ for yeast derivates, studies often refer to only one component (glucan or mannan) but usually contain both in various proportions.