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 appetence 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 consitent 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 $CD3^+CD4^+/CD3^+CD8^+$ 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 α-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 (*Cinnamonum 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 anti-microbial/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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Table 1. Effects of in-feed immunomodulators tested in post-weaned pig on various measures of performances, immune functions and health

	Weaning age (d)	Supplementation duration (wk)	Experimental conditions	Growth	Feed intake	G:F ratio	Immune traits	Health status	Reference
Yeast derivates									
Glucans[A]	14	4	S	0	0	0	n.d.	n.d.	Dritz *et al.*, 1995
Glucans[A]	21	4	S	0	—	0	0	n.d.	Dritz *et al.*, 1995
Glucans[A]	21	4	S	—	0	0	0	0	Kim *et al.*, 2000
Glucans[A]	26	4	S	Ê	0	0	0	0	Decuypere *et al.*, 1998
Glucans[B]	27-30	4	S	0	0	0	*Blood*: ↑ IgA, ↓ IgG	0	Sauerwein *et al.*, 2007
Glucans[C]	28	5	S	Ê	Ê	0	0	0	Li *et al.*, 2006
Glucans[D]	14	2	Ch	Ê	n.d.	n.d.	*Blood*: ↓ granulocyte and lymphocyte counts *Spleen*: ↑ TNF-α and IL-1β Ra mRNA	n.d.	Eicher *et al.*, 2006
Glucans[A]	18	6	Ch	—	n.d.	n.d.	*Blood*: ↓ haptoglobin	Increase sensitivity to *S. suis* infection	Dritz *et al.*, 1995
Glucans[C]	28	4	Ch	n.d.	n.d.	n.d.	*Plasma*: ↓ IL-6 and TNF-α, ↑ IL-10, ↑ IGF-1	n.d.	Li *et al.*, 2006
Glucans[E]	n.i.	8	Vac	0	0	0	*Blood*: ↓ specific Ab titer, ↑ $CD4^+$ and $CD8^+$ lymphocytes	n.d.	Hahn *et al.*, 2006
Glucans[B]	28	4	Vac	0	Ê	0	0	n.d.	Hiss and Sauerwein, 2003
Glucans[C]	28	4	Ch + Vac	n.d.	n.d.	n.d.	*Blood*: ↑ specific Ab titer, ↓ IL-6, ↓ TNF-α, ↑ IL-10	n.d.	Li *et al.*, 2005
Mannans[F]	18	5	S	Ê	Ê	Ê	0	n.d.	Davis *et al.*, 2002
Mannans[F]	19	4-5	S	Ê	0	Ê	*Lamina propria*: ↑ macrophage phagocytosis	n.d.	Davis *et al.*, 2004 (a,b)

Mannans[F]	21	4	S	0	0	0	0	0	Kim *et al.*, 2000
Mannans[G]	22	4	S	—	—	0	*Blood*: ↑ IgG	n.d.	White *et al.*, 2002
Mannans[H]	28	5	S	Ê	0	0	*Intestine*: ↓ IEL	n.d.	Lizardo *et al.*, 2008
Mannans[G]	11	7,5	Ch	0	0	0	0	0	White *et al.*, 2002
Mannans[H]	?	4	Ch	0	0	Ê	*Blood*: ↑ haptoglobin	0	Burkey *et al.*, 2004
Plant extracts*									
Thymol	24	3,5	Ch	0	—	Ê	*Blood*: ↑ IgA, ↑ IgM *Stomach*: ↓ TNF-α mRNA	0	Trevisi *et al.*, 2007
Thymol/ Carvacrol	n.i.	5	S	n.d.	n.d.	n.d.	0	n.d.	Muhl and Liebert, 2007
Carvacrol/ Cinnamaldehyde/ Capsicum oleoresin	20	3	S	0	0	0	*Jejunum*: ↓ IEL *Colon*: ↑ *lamina propria* lymphocytes *Lymph node*: ↓ of percentage of B lymphocyte	n.d.	Manzanilla *et al.*, 2006; Nofrarias *et al.*, 2006
Glucans	28	4	Ch	Ê	0	0	*Blood*: ↑ cortisol, ↑ IGF-1, ↑ lymphocyte proliferation	n.d.	Mao *et al.*, 2005
Glucans	28	3	Vac	Ê	Ê	0	*Blood*: ↑ WBC count (lymphocyte), ↑ lymphocyte proliferation, ↑ IL-2, ↑ IFN-γ	n.d.	Yuan *et al.*, 2006
Echinacea	n.i.	6	S	0	0	Ê	0	0	Maass *et al.*, 2005
Daidzein	11	6	Ch	Ê	Ê	Ê	*Spleen*: ↑ weight	0	Greiner *et al.*, 2001b
Genistein	10	5	Ch	0	Ê	0	*Blood*: ↓ IFN-γ *Spleen*: ↑ weight	↓ viremia	Greiner *et al.*, 2001a
Quillaja	29	3	S	0	Ê	—	*Blood*: ↑ IgG, ↑ IgA, ↑ C-reactive protein	0	Ilsley *et al.*, 2005
Quillaja	24	4	Ch	0	0	—	0	0	Turner *et al.*, 2002b
Ascophyllum	24	4	Ch	0	Ê	—	0	0	Turner *et al.*, 2002a
Animal by-products									
SDP	14	2	S	Ê	Ê	Ê	*Jejunum*: ↓ *lamina propria* cell density	n.d.	Jiang *et al.*, 2000
SDP[I]	20	3	S	0	0	Ê	*Ileum*: ↓ *lamina propria* lymphocyte density *Colon*: ↓ *lamina propria* lymphocyte density, ↓ IEL	0	Nofrarias *et al.*, 2006

SDP[I]	20	3	S	0	n.d.	Ê	*Jejunum, colon*: ↓ IEL	n.d.	Nofrarias *et al.*, 2007
SDP	14	1	Ch	0	n.d.	n.d.	*Spleen, thymus*: ↓ IL-1β, IL-6, TNF-α mRNA *Blood*: ↑ IFN-γ, ↑ TNF-α	Intestinal damage	Touchette *et al.*, 2002
SDP	17	2	Ch	0	0	▬	*Blood*: ↓ C-reactive protein, ↑ cortisol, ↑ IL-1β, ↑ IL-6	n.d.	Frank *et al.*, 2003
SDP[I]	17	1.5	Ch	0	0	0	*Blood*: ↓ IGF-1	Prevent diarrhoea	Yi *et al.*, 2005
SDP	21	2	Ch	Ê	Ê	Ê	*Intestine*: ↓ IL-8, TNF-α mRNA *Blood*: ↓ IgA	Prevent jejunal lesions (ulcer, oedema…) to ETEC	Bosi *et al.*, 2004
SDP	21	2	Ch	Ê	n.d.	n.d.	0	Prevent ETEC excretion and diarrhoea	Niewold *et al.*, 2007
Bovine colostrum	21	3	S	0	0	n.d.	*iPP*: ↓ B cell and ↑ T cell populations, ↑ lymphocyte proliferation, ↑ IL-4, IL-10 and IL-12 mRNA, ↓ IFN-γ mRNA *MLN*: ↑ lymphocyte proliferation, ↑ IL-2, IL-10 and IL-12 mRNA *Blood*: ↑ IgA, ↑ lymphocyte proliferation	n.d.	Boudry *et al.*, 2007
Lactoferrin	28	4	S	0	0	0	*Blood*: ↑ IgG, ↑ IgA, ↑ IgM, ↑ C4, ↑ IL-2, ↑ lymphocyte proliferation	Prevent diarrhoea	Shan *et al.*, 2007

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; Ê 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.