The Role of Immunostimulants in Monogastric Animal and Fish - Review -

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ABSTRACT: Many immunostimulating substances have been developed to improve immunity of domestic animals, although their exact mode of action and effects are not clearly defined, and they are now widely used in feed industry. Bacterial lipopolysaccharides, called endotoxin, in particular may have a profound effect not only on the immune system but also on macrophages of the reticuloendothelial system. Glucans from a variety of yeast cell wall have been shown to stimulate both specific and non-specific immune responses and to increase growth performance in pigs. Recently, there has been great interest in the role of complex carbohydrates in disease prevention and treatment. Mannanoligosaccharide is a glucomannanprotein complex derived from the cell wall of yeast. Generally, it was also known that the deficiencies of some major vitamins (vitamin A, E and C) and minerals (chromium and selenium) lead to impaired immune system and, as a result, immune function is depressed and recovery delayed. On the other hand, many researchers suggested that one possible reason for the superior performance observed in pigs fed plasma protein may be because of the presence of biologically active plasma proteins (e.g., immunoglobulins) which are known to contribute to the health of the starter pig. And, immunoglobulins present in plasma protein have been implicated as contributing to the overall immunocompetence of the newborn pig. Other immunostimulants, lactoferrin and lysozyme, mainly found in milk and egg white, have been known as having bactericidal and bacteriolytic effect. When considering practical use of immunostimulants, the concept of using immunostimulants is new to many people and, in most cases, it is poorly understood how and why such compounds act, and how they should be used in practice. Therefore, in order to clarify the reason for discrepancies in results, special attention should be paid to the dose/response relationship of immunostimulants and the duration of the effect. (Asian-Aus. J. Anim. Sci. 2000. Vol. 13, No. 8 : 1175-1187)

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INTRODUCTION

It is inevitable that intensive animal production stresses the animals by confinement, transport, handling, etc. and, as a result, creates a physiological condition characterized by suppressed immunity. During the evolution of animals, their immune systems have developed mechanisms to detect chemical structures which are typical for potentially dangerous microorganisms and use those structures as alarm signals to switch on the defense against infections. The immune system will therefore respond to immunostimulants as if challenged by a pathogenic microbe. Administration of an immunostimulant prior to an infection will elevate the defense barriers of the animal and thus provide protection against an otherwise severe or lethal infection. This paper was written to review the effects and the mode of action of some immunostimulants in domestic animals.

SOME COMMERCIAL IMMUNOSTIMULANTS

Immunostimulants are chemicals, drugs, streasors or actions that elevate the non-specific defense mechanism or the specific immune response (Anderson, 1992) and hence render animals more resistant to infections by viruses, bacteria, fungi and parasites. This definition describes the complexity of the interaction between the immune system and its environment. A number of studies have dealt with the evaluation of a number of different immunostimulants such as structural elements of bacteria (lipopolysaccharides, lipopeptides, glyco-proteins), various β-1,3-glucan products from bacteria and mycelial fungi, β-1,3/1,6-glucans from the cell wall of baker's yeast, complex carbohydrate structures (glycans) from various biological sources including seaweed, peptides in extracts of certain animals and made by enzymatic hydrolysis of fish protein, blood plasma, vitamins, minerals, and synthetic products.

SPECIFIC AND NON-SPECIFIC IMMUNITY

The mechanisms of the body that react passively or actively against foreign materials constitute the immune system. It represents a defense system to attack by harmful materials. Many factors contribute to the immune system in some form of protection against invading organisms. All animals keep their body free from harmful microorganisms and parasites by a defense system which consists of various types of leukocytes which act in concert with a number of
biochemical factors in the tissue fluids. The immune system functions at two levels which represent two defense lines against infectious agents. The first defense line is called non-specific immunity of the body because it is directed toward all potential intruders. The non-specific immune system consists of a battery of preformed antimicrobial substances and of phagocytic leukocytes, or phagocytes, which migrate toward the site of infection and there start to engulf and digest the infectious agent. Depending on their shape, microscopic structure and biological properties, the phagocytes are referred to as granulocytes, monocytes and macrophages. Non-specific immunity is also referred to as innate immunity because it is fully functional at birth in mammals. The second defense line is mobilized as a result of an infection and is called acquired or specific immunity. The infectious agent elicits the production of specific antibodies in a specialized group of leukocytes called lymphocytes. These antibodies are able to recognize and neutralize the same infectious agent if it enters the body later. The body thereby acquires a targeted, or specific disease resistance. During a period after birth of mammals, the specific immunity (ability to produce specific antibodies) is poorly developed. The formation of antibody producing cells in the gut of young mammals is apparently triggered by the microbial flora, which becomes established in early life, and the antibodies produced by cells in the intestinal tissues belong mainly to the IgA class which are the major local secretor; E. coli have been controlled by dietary treatment of the prepartum sow and post-weaning piglets (Porter et al., 1985). Similar techniques have been used in the control of salmonella in the calf (Balger et al., 1981) and coccidiosis in poultry (Davis et al., 1986).

Vaccines, which usually are preparations of killed or weakened pathogenic microorganisms, elicit the production of the same specific antibodies as those produced as the result of an infection by the same pathogen. Therefore, vaccination is a way to induce resistance to a specific disease. Unlike vaccines, immunostimulants elevate the overall resistance of animals to a number of infectious agents simultaneously.

The biological and cellular processes in the specific and the non-specific immune system are interwoven and highly interdependent. For instance, when an immunostimulant enhances the non-specific immune system by interacting with phagocytic cells, it will also activate processes in the specific immune system. This is why compounds with immunostimulating properties are used as adjuvants in vaccines to increase antibody production and vaccine efficacy. Fishes have both non-specific and specific immunity but the latter is less complex than in mammals and probably of less relative importance. Invertebrates such as shrimp lack a specific immune system similar to that of vertebrates and are apparently entirely dependant on non-specific immune mechanisms to resist infections (Soderhall and Cerenius, 1992)

**THE MODE OF ACTION OF IMMUNOSTIMULANTS**

The mode of action at a molecular level is known only for a few immunostimulants. The various immunostimulants of bacterial origin are fragments of the cell structures with very complex chemical structure. Such materials interact directly with phagocytic leukocytes as well as with the leukocytes involved in antibody production. Immunostimulants of bacterial origin therefore seem to cause a general enhancement of many different immune reactions in the body and they are also antigenic in nature, inducing antibody production against the immunostimulant itself. However, bacterial immunostimulants, used as adjuvants in vaccines have the disadvantage that much of the antibody producing capability of the body is wasted on the adjuvant.

**Bacterial lipopolysaccharides (LPS)**

Microbial gut flora are known to influence immunity in a variety of ways and bacterial lipopolysaccharides in particular may have a profound effect not only on the immune system but also on macrophages of the reticuloendothelial system. Bacterial lipopolysaccharides, called endotoxin, are among the most potent immunostimulants known today. However, LPS have no relevance as immunostimulants for practical use in mammals because they are at the same time very toxic materials which cause inflammation reactions resulting in fever, reduced feed intake and impaired performance of animals; however, fish and shrimp are less sensitive to LPS. The toxic effects of LPS are caused by cytokines, which are produced when leukocytes, primarily macrophages, are stimulated by LPS.

Although LPS has traditionally been regarded as a B cell mitogen and macrophage activator, it is now apparent that an interaction between T cells and LPS occurs (Motta et al., 1986). In particular, bacterial endotoxin LPS potentiates antigen specific proliferation of T helper cells. This effect is independent of interleukin I action, the T cell activation factor released by macrophages after LPS stimulation. The prospect thus emerges of multiple modulatory effects of bacterial endotoxin on gut immune responses. Not only could induction of tolerance be enhanced by selective increases of suppressor cell activity, but by polyclonal activation of B cell responses and increases in T cell help, both specific and non-specific
protective immune responses could be improved.

**β-Glucan**

Glucans have probably attracted the attention of researchers more than any other immunostimulants. Glucans are used either by direct injection into the body or by oral administration through the feed. Glucans from a variety of yeasts cell wall have been shown to stimulate both specific and non-specific immune responses (Williams et al., 1989; Robertsen et al., 1990; Suzuki et al., 1990; Chen and Ainsworth, 1992; Jeney and Anderson, 1993; Jørgensen et al., 1993) and to increase growth performance in pigs (Schoenherr et al., 1994). A pure β-1,3/1,6-glucan interacts very selectively with specific receptors on the macrophages, granulocytes, and NK-cells and as a result, these are the only cells which are activated directly (Engstad, 1994; Engstad and Robertsen, 1993, 1994). These cells produce cytokines, which stimulate T-lymphocytes and in turn B-lymphocytes which therefore become more active in producing antibodies against bacteria and viruses. This is why β-1,3/1,6-glucans not only elevate the nonspecific defense barriers of animals but also enhance the specific resistance to environmental pathogens, and make vaccines more potent. Despite the fact that β-1,3/1,6-glucans activate macrophages and hence induce cytokine production by these cells, β-1,3/1,6-glucans do not negatively affect growth and appetite, unlike lipopolysaccharides. A lot of scientific research data have shown that β-1,3/1,6-glucans stimulate growth of fish (Onarheim, 1992), shrimp (Sung et al., 1994), pigs (Schoenherr et al., 1994; Dritz et al., 1995; Boddez, 1998), and reduce diarrhea and mortality in weanling pigs (Kim et al., 1999). Growth enhancement may partly be due to improved nonspecific immunity and hence reduced bacterial stress on the animals. It has been demonstrated that a β-1,3/1,6-glucan administrated by the oral route causes increased resistance in mice (Nicoletti et al., 1998). Weaned pigs receiving the β-1,3/1,6-glucan in their diet grew faster than pigs fed control diet without glucan (Schoenherr, 1994). Addition of β-1,3/1,6-glucan in sow diets stimulated the secretion of antibodies in the milk (Boddez, 1998). Oral β-1,3/1,6-glucan enhanced growth and macrophage activity in pigs (Dritz et al., 1995). The possible mode of action of β-glucan in weaned pigs are as follows; 1) growth enhancements by β-glucan could be the result of an increase in nonspecific immunity, 2) the positive results of feeding β-glucan to weaned pigs could be evoked by eliciting specific immune reaction, 3) β-glucan could decrease inflammatory cytokines responses (Poutsiaka et al., 1993), thereby allowing nutrients to be partitioned toward growth (Klasing et al., 1987), 4) β-glucan in the weaning pig diets might abate soybean induced hypersensitivity by enhancing tolerance to soybean proteins.

Recently it has been demonstrated that feeding of coho salmon with β-1,3/1,6-glucan in combination with vitamin C induces a very efficient protection against bacterial disease. Challenge experiments carried out with salmon in a governmental fish disease laboratory in Norway confirmed that β-1,3/1,6-glucan enhances disease resistance when administered in the feed (Raa et al., 1992). It was also demonstrated that feeding of β-1,3/1,6-glucan prior to vaccination improved the efficacy of a vaccine against *Vibrio salmonicida* (Raa et al., 1992). Seto (1994) reported that with the addition of β-1,3/1,6-glucan in the feed for yellowtail, the mortality during a natural disease outbreak in a commercial sea farm was markedly reduced.

**Mannanoligosaccharides**

The interaction between nutrition and disease has long been known. Not only is the food we eat important to health by providing the building blocks of the immune system, but certain compounds that we eat may help immunity directly. Recently, there has been great interest in the role of complex carbohydrates in disease prevention and treatment. One of the most fascinating of these oligosaccharides is mannanoligosaccharide. Mannanoligosaccharide is a glucomananoprotein complex derived from the cell wall of yeast. The activities of this compound are absorption of enteric pathogens and immunomodulation. Absorption of pathogenic bacteria has been explained by several scientists over the past 15 years. Certain components of bacterial surfaces called lectins are involved in the onset of enteric and urinary disease by mediating adherence of the bacteria to epithelial cells in these sites (Beachey, 1981; Firon et al., 1987; Roberts, 1984). Although there are a number of lectins that are specific for sugars such as galactose and fructose, lectins specific for mannose predominate in intestinal pathogens. These lectins are located on the exterior of the cell and are associated with pili or fimbriae of bacteria. Bacterial cells with pili that are specific for mannose attach to mannose containing cells in the intestinal tract. Once these cells are attached, they can then colonize the tract and cause disease. Mannanoligosaccharide provides a mannose rich source for attachment which will adsorb bacteria that would otherwise attach to the gut wall. Since mannanoligosaccharide is not degraded by digestive enzymes, it passes through the tract with the pathogens attached, preventing colonization. Several studies have been conducted on the role of mannans and their derivatives on the binding of pathogens to epithelial cells in the gastrointestinal tract. *E. coli* with mannose was present (Salit and Gotschlich, 1977).
Because of the major role of mannanoligosaccharide involving interference with colonization and adherence of intestinal pathogens, mannanoligosaccharide was regarded as improving growth performance (Kim et al., 1999), reducing diarrhea (Kim et al., 1999) and mortality in pigs (Kim et al., 1999) and broilers (MacDonald, 1996).

Except the role of mannanoligosaccharide on blocking colonization of pathogen, many researchers have investigated the immunostimulatory properties of mannanoligosaccharide. Sisak (1994) and Zenkoh (1995) have determined increased activity of phagocytic cells from mice fed mannanoligosaccharide. Savage and Zakrezevska (1996) reported increased concentration of IgG and IgA with mannanoligosaccharide in an in vitro trial. According to the study conducted by Spring and Privulescu (1998), mannanoligosaccharide increased the reactivity of the intestinal lymphocyte and the phagocytic ability of the white cells in the intestine. They also reported that pigs fed diet supplemented mannanoligosaccharide showed enhanced levels of interleukin-2 (IL-2), which is known as a growth factor for T-cell and is required for T-cell proliferation and differentiation.

In conclusion, the effects of mannanoligosaccharide on immune response starts with inhibiting the attachment of microorganisms to the cell surface, which may impair immune response. Thus, metabolic changes that occur successively after immunological stress can be prevented.

**Vitamins**

Severe disease often rapidly leads to a suboptimal status, or deficiency, of certain vitamins, especially in the first weeks of life when reserves are still low. As a result, immune function is depressed and recovery delayed. At this age the immune system is maturing and requires adequate supplies of all vitamins.

**Vitamin A** is essential for normal vision, growth and epithelial cell differentiation, bone development and reproduction (Bridges, 1984; Roberts and Sporn, 1984; Dennert, 1984). Vitamin A deficiency and excess is associated with depressed immunity (Friedman and Sklan, 1989a; Shapiro and Edelson, 1985). Deficiency is accompanied by low levels of serum immunoglobulins (Leutskaya and Fais, 1977; Harmon et al., 1963; Sirisinha et al., 1980), impaired immunoglobulin IgG (Barnett, 1983; Smith et al., 1987) and Ig A responses (Sirisinha et al., 1980), reduction in delayed-type hypersensitivity reactions (Ulur et al., 1963; Athanassiades, 1981; Smith et al., 1987), depressed responses to mitogens (Nauss et al., 1979; Davis and Sell, 1983) and reduced natural killer cell activity (Nauss and Newberne, 1985). Excess vitamin A decreased immune responsiveness in chicks to defined protein antigens (Friedman and Sklan, 1989b) and to *Escherichia coli* (Friedman et al., 1991) and high intakes of vitamin A were reported to decrease cellular immune functions in mice (Dennert and Lotan, 1978).

β-carotene may also have an independent effect on immune responses, separate from its provitamin A activity. β-carotene and other carotenoids with nine or more conjugated double bonds may enhance immune function by quenching single oxygen and other reactive oxygen species, including free radicals. Vitamin A, in contrast, cannot quench single oxygen and is a relatively poor antioxidant (Burton and Ingold, 1984; Goodwin, 1984). β-carotene can protect phagocytic cells from autooxidative damage, enhance T and B lymphocyte proliferative responses, stimulate effector T cell functions, and enhance macrophage, cytotoxic T cell and natural killer cell tumoricidal capacities, as well as increase the production of certain interleukins (Bendich, 1989).

**Vitamin E** and ascorbic acid work closely together in stimulating the function of the immune system. In leucocytes, vitamin E plays a major role in maintaining the stability of the arachidonic acid contained in large amounts in membrane lipids. Under the effects of cyclooxygenase and lipoxygenase, this essential fatty acid is converted respectively into prostaglandins and leukotrienes, substances importantly involved in the inflammatory response.

Vitamin E homologues inhibit the activity of phospholipase A2, which breaks down lipids to release arachidonic acid for prostaglandin and leukotriene synthesis. In vitamin E deficiency, the production of inflammatory mediators, such as prostaglandin E2 and leukotriene B4, is increased.

Vitamin E prevents excessive activity of superoxide anions and hydrogen peroxide in phagocytes, and prolongs the functional life of the cells. In activated murine macrophages, addition of tocopherol succinate produced a 3.8 fold rise in interleukin 1 production. Retrovirus-induced immunosuppression in the chicken can be prevented by vitamin E administration (Romach et al., 1993). In vitamin E deficiency, lipid peroxide production in neutrophil membranes increases 1.5-fold. The cells release more hydrogen peroxide and their life span is shortened. Phagocytosis of immunoglobulin-bacterium complexes is reduced. In a study of sows fed a diet containing only 0.29 mg vitamin E per kg from the time of insemination until day 4 postpartum, neutrophils showed decreased yeast-cell phagocytosis and bactericidal activity on day 90. A feed with an adequate vitamin E content (60 mg/kg) but low in selenium (89 g/kg) had the same effect (Kuryastuti et al., 1973). Vitamin E plays a role in T-cell differentiation in the thymus. Deficiency during the
growth phase is associated with a fall in helper T-cell numbers and rise in suppressor T-cell numbers in the blood. The production of interleukin 2 in activated T helper cells is reduced in vitamin E deficiency, and that of PGE₂ increased (Moriguchi et al., 1994). In bulls, supplementation of a vitamin E-deficient ration with vitamin E 1,000 IU daily raised the leukocyte concentration of mRNA for interleukin 1 by 55%.

Ascorbic acid (vitamin C) plays a major role in activating and maintaining the function of phagocytes. In deficiency, neutrophil motility is decreased, and hence the speed with which these cells migrate to inflamed regions also decreased. A high ascorbic acid concentration is found in the cells of the adrenal cortex, where it plays a role in regulating glucocorticoid (cortisol) secretion. In stress states, particularly fever, ascorbic acid consumption increases. At sufficiently low plasma levels there is decreased uptake by leukocytes, which require large amounts for their functioning. When ascorbic acid is added to the feed of monogastric animals, its plasma concentration rises, which in various species stimulates uptake by the cells of the adrenal cortex. This reduces glucocorticoid secretion, with resultant beneficial effects on growth and productivity. The addition of 1,000 mg ascorbic acid per kg of feed in chickens reduced the immunosuppressive effect of four intramuscular injections of 2 mg hydrocortisone (Pardue and Traxton, 1984).

Lymphocyte proliferation following mitogenic activation depends on physiological plasma levels of ascorbic acid. In chickens fed ascorbic acid supplements (500 mg/kg) for 7-49 days after hatching and inoculated with Newcastle-disease-virus vaccine or pasteurella anatipesifer on day 21, high plasma antibody levels and marked lymphocyte proliferation were observed on day 28 even in those supplemented for only 7 and 14 days. As an explanation for this effect it is suggested that ascorbic acid supplementation in the first week after hatching promotes maturation of the immune system (Franchini et al., 1994). The dependence of immune function on ascorbic acid supply has also been demonstrated in guinea pigs. In one study the animals were immunized with a tuberculin preparation three weeks before starting an ascorbic acid free diet. The reaction to intradermal tuberculin after four weeks of deficiency was only 10% of that in control animals receiving daily supplements of 25 mg per kg body weight. The number of antibody producing cells in the spleen and splenic lymphocyte responding to stimulation were respectively only 13% and 28% of the control values. After ascorbic acid supplementation (25 mg/kg daily) the immune system recovered within eight weeks (Thurman and Goldstein, 1979).

Minerals

For some minerals a deficient status leads to increased susceptibility to infection. Although many generalized functions for some nutrients could lead to altered immune function, specific functions for some minerals in immunity are not yet identified.

Chromium (Cr) is well known as an essential trace element for humans and laboratory animals. Chromium may have several biological functions, including roles in nuclear protein and RNA synthesis, but its predominant physiological role seems to be an integral component of glucose tolerance factor (GTF) to potentiate the action of insulin (Anderson and Mertz, 1977). Chang and Mowat (1992) have noted an interaction between Cr and an antibiotic. In their initial studies, improvements in weight gain by calves during the first 28 days after arrival were comparable between supplemental Cr and long acting injectable oxytetracycline. In steers without preventive or therapeutic antibiotic, Cr increased body weight gain by 52%. In the presence of antibiotic, Cr had no positive effect on performance. The main reason for this large improvement was the marked reduction in number of sick calves. In their initial studies, Chang and Mowat (1992) demonstrated that supplemental Cr increased serum immunoglobulin levels in calves given corn silage plus soybean meal during the growing period. Moonse Shageer and Mowat (1993) reported that during the stress period Cr reduced rectal temperature and increased antibody titers to injected human erythrocytes. Dairy cows receiving supplemental chelated Cr during early lactation period also had higher antibody responses to a variety of test antigens and superior cell-mediated immunity following in vitro stimulation with a T-cell mitogen (Burton et al., 1993). It is clear that organically complexed Cr is a potent immunomodulator of specific immune responses in cattle. Chang and Mowat (1992) reported that supplemental Cr from high Cr yeast decreased serum cortisol of growing steers. Various stressors are known to increase serum cortisol. Glucocorticoids are known to suppress the immune system (Munck et al., 1984). Decreased cortisol may have caused the increased immunoglobulin levels noted in steers supplemented with Cr and soybean meal. Decreased serum cortisol with Cr supplementation could have particular implications for the high producing dairy cow. Cortisol has been reported to be antagonistic to milk production (Sartin et al., 1988). Moreover, increased cortisol concentrations associated with physiological stress may negatively affect estrus cyclicity in high producing dairy cows (Vighio and Liptap, 1990). Subiyatno et al. (1993) reported that supplemental Cr for prepartum primiparous and postpartum cows reduced serum cortisol.
Selenium is one of the most important minerals in animal diets. Selenium deficiency is associated with diminished activity of selenium dependent glutathione peroxidase in neutrophils, and negatively affects neutrophil bacterial activity. Lymphocyte proliferative capacity decreases in various animal species. Reproductive benefits have been observed from supplemental vitamin E and selenium by improvement of herd health and sows’ immune response. Whitehair and Miller (1985) demonstrated that diets low in Se and vitamin E precipitated a high occurrence of MMA, whereas when these nutrients were supplemented, the disease malady was eliminated.

The effectiveness of both vitamin E and selenium in increasing the humoral immunogenic response in pigs was demonstrated by Peplowski et al. (1980). Cellular immunity also appears to be enhanced from the combination of both nutrients. Larson and Tollersud (1981) reported that pig lymphocytes responded to both Se and vitamin E in a phytohaemaglutinin response. Wuryastuti et al. (1993) demonstrated that pregnant gilt at 90 days of postcoitum and (or) within 3 days postpartum had improved cellular immune responses when supplemental vitamin E and Se (0.3 ppm) were jointly provided.

Blood plasma
Blood plasma collected from pigs, cattle and chickens contains relatively high concentrations of proteins, particularly immunoglobulins. Immunoglobulins are proteins that function to inactivate or destroy antigens in the body. Dietary additions of spray-dried plasma proteins result in improved daily gains and efficiency of feed utilization in antigen-challenged (high immune system) pigs but not in animals with a low degree of antigens (low immune system) exposure. The major protein fractions of plasma include high molecular weight protein (globulin), medium molecular weight protein (albumin) and low molecular weight components. Can (1995) reported that pigs fed the diet containing plasma grew faster and consumed more feed during the first 2 weeks postweaning than pigs fed the control diet. Growth performance of pigs fed the high molecular weight fraction was similar to pigs fed plasma, while growth of pigs fed the medium or low molecular weight fraction was not different from the negative control. Furthermore, villus surface area as well as the activity of lactase and maltase in the jejunum was increased in pigs fed the high molecular weight fraction in plasma compared to the negative control. Other scientists have also reported that the positive response of the plasma could be achieved by feeding the high molecular weight fraction (Owen et al., 1995; Pierce et al., 1995, 1996; Weaver et al., 1995). Hansen et al. (1993) also reported that a possible reason for the superior performance observed in pigs fed plasma protein may be because of the presence of biologically active plasma proteins (e.g., immunoglobulins) that may contribute to health of the starter pig. Immunoglobulins present in plasma protein have been implicated as contributing to the overall immunocompetence of the newborn pig (Gatnau et al., 1989). Thus, these data support the hypothesis that the high molecular weight fraction present in plasma has a biological function and influences performance in the pigs.

Lactoferrin and lysozyme
The biological significance of the proteins, lactoferrin and lysozyme relates to their protective roles in a variety of bodily secretions, especially milk. These non-antibody protective proteins augment and complement the production of antibodies by the immune system. The concentrations of these milk proteins in milk vary according to species. Lactoferrin is an iron-binding protein found in human milk and the milk of all mammalian species, and is also found in saliva, tears, bronchial and nasal secretions and pancreatic fluid and indeed any other bodily secretion. Lactoferrin displays both bacteriocidal and bacteriostatic effects. In terms of the bacteriocidal effects, lactoferrin has the ability to kill species of bacteria, which action is not associated with its ability to compete with the bacteria for iron.

Lysozyme, which is a strong bacteriolytic agent, has been demonstrated in a variety of tissue secretions and is found in large quantities in egg white. It is also present at high concentrations in human milk but at lower concentrations in bovine milk. Lysozyme has the ability, as an enzyme, to bring about the breakdown of bacterial cell wall and so destroy susceptible bacterial cells due to osmotic factors.

THE PRACTICAL USE OF IMMUNOSTIMULANTS
Immunostimulants may be used prior to situations known to result in stress and impaired performance of animals or when there is a risk of increased exposure to pathogenic microorganisms and parasites. Immunostimulants are primarily prophylactic agents. Therefore, they should not be used to combat a disease, which has already broken out. The concept of using immunostimulants, which increase the level of resistance to all potential infectious agents simultaneously, is met with enthusiasm and curiosity in the field of animal husbandry. The concept of using immunostimulants is new to many people and in most cases it is poorly understood how and why such compounds act, and how they should be used in practice. The mode of action of immunostimulants is still not clear. The practitioners in the field have experienced variable results. In order to clarify the
reason for discrepancies in results, special attention should be paid to the dose/response relationship of immunostimulants and the duration of the effect. The general immunological status of the animals may affect the results. Unlike many chemotherapeutics, immunostimulants do not show a linear dose/response relationship (Bliznakov and Adler, 1972; Olivier et al., 1985; Yano et al., 1989; Roberts et al., 1990; Anderson, 1992; Shoehnerr et al., 1994; Sung et al., 1994). Discrepancies and disagreements in the literature regarding efficacy of certain immunostimulant preparations may be due to differences in doses. Moreover the time span between administration of a given immunostimulant and maximum effect varies with the dose. Therefore, the comparison of results from different laboratories may be difficult. An immunostimulant may also be beneficial if it is used prior to, or during development phases when animals are particularly susceptible to infectious diseases. Experiments with animals indicate that enhanced disease resistance may build up just after administration of an immunostimulant. For instance a nasal spray containing the β-1,3-glucan given to mice provided almost complete protection when given only 24 hours prior to a challenge with a lethal influenza virus (Maeda et al., 1994).

It has been shown that immunostimulants (β-1,3-glucan) may act in synergy with antibiotics in preventing infections in human patients (de Felippe et al., 1993) and fish in aquaculture (Thompson et al., 1993). There is a sound biological basis for such a synergy, that is to say, the aggressiveness of the infectious bacteria is dampened by the antibiotics while the body’s own antibacterial mechanisms are stimulated. It is therefore an attractive option to use immunostimulants in combination with antibiotics when a disease outbreak is in early phase, or prior to periods when the need to use antibiotics is anticipated. During an infection the balance between the invasive process of the pathogen and the defence reactions of the host is shifting in favor of the pathogen. Antibiotics are used to change this balance to favor the host by inhibiting growth or killing the pathogen. The effect of an antibiotic is attained only when the host has a functional immune system. If the system is suppressed or damaged the use of antibiotics will be of marginal importance and only postpone the final outcome. On the other hand, activation of the immune system prior to or during an infection may potentiate the action of an antibiotic. This hypothesis is accordance with the observations by Dietrich et al. (1981) who showed that an immunostimulatory muramyl dipeptide potentiated the effect of antibiotics in experimentally infected mice.

The development of vaccines against a virus is time consuming and expensive, and the costs will be high to develop efficient products against the great number of different viral diseases which may affect the many different cultured species. Therefore, it is a wise strategy to reduce the risk of virus infections by combining good facilities and high quality feeds with the use of immunostimulants.

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