# Rationale for Using Immunopotentiators in Domestic Food Animals

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## I. Introduction

Prevention and treatment of disease are primary concerns of everyone involved in the production of domestic food animals. Producers, veterinarians, and production animal specialists, such as nutritionists and reproductive physiologists, can all cite specific economic endpoints that are directly related to the incidence and intensity of disease in food production animals. Indeed, even the consumer of animal agricultural products is greatly affected by the cost of maintaining an abundant supply of healthy food animal products. Consequently, much effort and expense are directed toward minimizing the incidence of disease in domestic food animals. One means of decreasing the impact of disease in food animals is increasing the animal's ability to withstand infections.

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Regulation of the immune response is extremely complex. Neverthe-less, we are slowly beginning to understand how the immune system, indeed the whole animal, orchestrates the body's response to an invad-ing pathogen. With the knowledge, however incomplete, of how the immune system responds to disease-causing organisms, we can devise ways of intervening in the regulation of the immune system, particu-larly by modulating the host's immune response.

Immunomodulation, as the term implies, can be used to designate either a suppression or an augmentation of an immune response. The necessity and capability of suppressing the function of the immune system are well recognized in such areas as organ transplantation and autoimmune disorders. However, in general, medically induced immuno-suppression is not a practical concern in domestic food animals. Conversely, augmentation of immunity has received much attention in domestic food animals and provides a means of increasing the host's resistance to disease. Various chemicals and biological substances have been used and evaluated as immunomodulators in domestic food animals and will be discussed in the following chapter of this book. Other synonyms for immunomodulators that are frequently used in-clude immunostimulators, immunopotentiators, immunotherapeutic agents, and biological response modifiers.

## II. Why Are Immunomodulators Needed?

Vaccination of domestic food animals against economically impor-tant pathogens is effective and has increased the efficiency of food animal production. However, even with the successes attained in food animal production through vaccination programs, tremendous eco-nomic losses still occur in animal agriculture that are directly related to the health of the animal. Two important examples of diseases that still cause large economic losses, bovine respiratory disease and mastitis, will be used to illustrate this point.

Respiratory disease of cattle continue to present a serious economic burden to the producer. The annual economic loss to the North Ameri-can cattle industry from bovine respiratory disease has been estimated to range from $250 million to $1 billion (Babiuk et al., 1987). The etiology of bovine respiratory disease is very complex and multifacto-rial; the interactions of viruses, bacteria, and stress greatly contribute to the disease process (Loan, 1984). Vaccines against viruses and bac-teria involved in bovine respiratory disease are available and used. However, bovine respiratory disease still accounts for 65% of the health problems and deaths among feedlot cattle (Edwards, 1987). In addition

to losses due to death, economic losses caused by bovine respiratory disease include reduced growth performance and increased treatment costs. These losses emphasize the need for alternative or complementary therapeutic approaches, such as immunomodulators, that may be well suited for the multifactorial etiology involved in the disease.

In economical terms, mastitis is the most devastating disease affecting dairy cows. In the United States, losses attributed to mastitis approach $2 billion each year; 70% of this economic loss is due to a reduced milk yield as a result of subclinical mastitis (National Mastitis Council, 1987). Similarly, a French epidemiological survey found that mastitis was by far the most frequent pathology affecting dairy cows (Barnouin *et al.*, 1986). Vaccination against bacteria that cause intramammary infections has been attempted as a means of decreasing mastitis. However, even in studies that have shown beneficial effects of immunization against mastitis, vaccination did not prevent new intramammary infections (Pankey *et al.*, 1985). Antibiotic therapy is used in the control of mastitis. However, because *Staphylococcus aureus* mastitis responds poorly to antibiotic therapy and because of the problem of antibiotic residues in milk, the effectiveness of antibiotic therapy in mastitis prevention and treatment is limited.

These specific examples emphasize the need to continue to search for more effective ways to minimize the impact of disease on animal production. Augmentation of the animal's immune response with the intent of increasing resistance to disease-causing organisms should decrease the economic loss due to disease in food animal production. Immunomodulation may provide an effective means of enhancing the ability of domestic food animals to withstand disease.

### III.  Specific versus Nonspecific Immunomodulation

When one considers the possibility of enhancing an animal's immune response, a question that must be addressed is whether specific or nonspecific immunomodulation is desired or required. Specific immunomodulation involves the potentiation of the host's immune system toward a unique, specific antigen. Vaccination programs are perhaps the best example of producing specific immunity in domestic food animals. Nonspecific immunomodulation generally is an attempt to heighten immunologic capabilities at a time when an animal may be exposed to one or several pathogens and/or be immunocompromised. Both of these concepts will be discussed further.

The distinction between adjuvants and specific immunomodulators is

blurred and may be only a matter of semantics. Classical and new adjuvants offer the capability of enhancing specific immunity and are discussed in great detail in Chapter 5 of this volume. However, some substances that are not generally thought of as adjuvants, such as the interleukins and interferons, also induce a state of specific immuno-modulation. For example, peripheral blood mononuclear cells from cattle injected with recombinant bovine interleukin-2 display enhanced cytolytic capabilities against bovine herpesvirus-infected target cells (Reddy *et al.*, 1989a). However, protection against a bovine herpesvirus challenge was observed only in animals that received a vaccination against the virus in conjunction with injections of interleukin-2. Thus, in this case both nonspecific and specific immunomodulation was produced in cattle that were administered interleukin-2, but only specific immunomodulation resulted in protection against a viral challenge.

Theoretically, the capability of potentiating the host's immune response at a time when it might be immature, compromised, or overcome with pathogens should enhance the animal's ability to resist disease. This is the rationale for nonspecifically augmenting an animal's immune response. Nonspecific immunomodulation has potential in at least 3 different conditions: (1) during the neonatal period when the immune system may not be fully developed; (2) during periods of stress-induced immunosuppression; and (3) during virus- or bacteria-induced immunosuppression.


## A. THE NEONATAL PERIOD

Because of a very efficient placental barrier, pig, horse, and ruminant fetuses are generally very well protected from *in utero* antigenic stimuli. Therefore, although fully immunocompetent at birth, domestic food animal newborns differ from other mammalian neonates in being immunologically "virgin" (Kim, 1975; Salmon, 1984) and the development of totally effective immune defenses requires 2 to 3 weeks. During this critical neonatal period the young animal is highly susceptible to microbial infections.

Postnatal development of immune functions has been most extensively studied in the pig (Sterzl and Silverstein, 1967; Kim, 1975). Most immune parameters that have been studied appeared to be very low at birth and reached adult values at about 1 month of age. Thus, the percentage of T and B lymphocytes in peripheral blood, as estimated by E-rosettes and anti-Ig immunofluorescence techniques, was shown to

increase from 3 to 4% at birth to adult values by 35 days of age (Reyero *et al.*, 1978). A similar age-related increase has been described for serum concentrations of the third component of complement (C3) in pigs (Tyler *et al.*, 1988).

Because of the high incidence and economic impact of respiratory and intestinal infections in young domestic animals, it is important to review studies related to the postnatal development of the mucosa-associated immune system in the pig. At birth, the intestinal, nasal, and tracheobronchial mucosa are devoid of plasma cells. Plasma cells first appear in the respiratory tract at 6–7 days of age and reach adult values at 3–4 weeks of age. This postnatal development was described for cells containing IgA as well as IgM and IgG (Bradley *et al.*, 1976). In the intestinal lamina propria, cells with cytoplasmic IgM appeared at 4–5 days after birth, earlier than the plasma cells containing IgG and IgA. In immunologically mature pigs, IgA plasma cells predominate, however in young animals the predominant isotype secreted by lamina propria plasma cells is IgM, and adult values are not attained until 4–9 weeks of age (Allen and Porter, 1977). Similarly, porcine gut-associated lymphoid tissue is poorly developed at birth and matures during the first month of life, showing an increase in number of small intestinal intraepithelial lymphocytes and the development in size and structure of the Peyer's patches (Chu *et al.*, 1979; Pabst *et al.*, 1988).

Inside the lung, residing at the air–tissue interface and directly exposed to inhaled microorganisms or air pollutants, the alveolar macrophage functions as the primary defense against respiratory infections (Hocking and Golde, 1979). Functional properties of alveolar macrophages, including their immunological and antiinfectious features, have been studied in domestic food animals (Khadom *et al.*, 1985; Charley, 1985). Rothlein *et al.* (1981) have studied the postnatal development of alveolar macrophages in Minnesota miniature swine. These researchers showed that lavage fluids from the lungs of newborn piglets were devoid of macrophages. However, within 2 to 3 days after birth, macrophages gradually appear inside the lung airspaces and adult values are reached at 2 weeks of age. Furthermore, macrophages collected from piglets less than 1 week old showed immature function, i.e., lower phagocytic capacity and enzyme content than adult cells. The postnatal development of lung macrophages appears to depend upon nonspecific antigenic stimulation since germ-free piglets have a much lower number of alveolar macrophages than specific-pathogen-free piglets (Rothlein *et al.*, 1981). Additionally, alveolar macrophages from young piglets have been shown to be more permissive to pseudorabies

virus, yielding higher virus progeny titers, than cells from older animals (Iglesias *et al.*, 1989).

A last example of an immune defect occurring during the neonatal period is given by studies on porcine natural killer (NK) cells. Natural killer cell activity in the peripheral blood of newborn pigs is much lower (often undetectable) than the activity of adult cells. This NK cell defect has been observed regardless of the target cell system used: human tumor cells (Huh *et al.*, 1981), virus-infected cells (Cepica and Derbyshire, 1984a; Yang and Schultz, 1986), or porcine tumor B-cells (Onizuka *et al.*, 1987). Of particular interest are the observations that postnatal development of NK cells activity, which requires 2–3 weeks in specific-pathogen-free miniature swine (Huh *et al.*, 1981) and in conventionally reared Large-White pigs (Charley *et al.*, 1985), is delayed in germ-free miniature piglets (Huh *et al.*, 1981). These data imply that microbial flora play a role in the maturation process of NK cell activity in neonates. Due to the high incidence of intestinal infections in young domestic food animals it is worth noting that porcine intestinal intraepithelial lymphocytes show high NK cell activity against transmissible gastroenteritis virus (TGEV)-infected pig kidney cells, whereas the same cells isolated in young piglets have no NK cell activity (Cepica and Derbyshire, 1984a). This observation has led to the hypothesis that a NK cell defect could in part explain the great susceptibility of piglets to coronavirus-induced transmissible gastroenteritis. Indeed, adoptive transfer of adult pig leukocytes established functional NK cell activity in recipient piglets and reduced their susceptibility to a TGEV challenge (Cepica and Derbyshire, 1984b).

The examples described above illustrate the existence of several different immune defects (see Table I) in neonatal domestic food animals. This lower functional immune status during the neonatal period could explain some of the neonates' susceptibility to infectious diseases, especially intestinal infections. Thus, the potential exists to increase the neonates' immune functions by using immunomodulators. A few studies have been conducted exploring means of enhancing the young animals' immune functions. For example, newborn piglets' NK cell activity was shown to be responsive *in vitro* to interferon (Charley *et al.*, 1985), and *in vivo* to poly I: C (Lesnick and Derbyshire, 1988) or bacterial extracts (Kim, 1984). Additionally, isoprinosine has been shown to enhance the immunocompromised immune status of artificially reared neonatal pigs (Hennessy *et al.*, 1987). In the following chapters several immunomodulating strategies will be reviewed and should help to define possible immunotherapeutic approaches to enhance young domestic food animals' resistance to disease.

TABLE I

IMMUNOLOGICAL IMPAIRMENT OF NEWBORN PIGLETS: A SUMMARY OF IMMATURE
IMMUNE FUNCTIONS

| Immunological compartment | Nature of the immune "defect" | Reference |
|---|---|---|
| Blood | Low percentage of T and B lymphocytes | Reyero et al. (1978) |
| | Low concentration of C3 | Tyler et al. (1988) |
| | Low primary antibody response | Metzger et al. (1978) |
| | Low NK cell activity | Huh et al. (1981); Cepica and Derbyshire (1984a) |
| Intestine | Low number of plasma cells | Allen and Porter (1977) |
| | Low number of intraepithelial lymphocytes | Chu et al. (1979) |
| | Low number of Peyer's patches | Pabst et al. (1988) |
| | Low NK cell activity | Cepica and Derbyshire (1984b) |
| Lung | Low number of plasma cells | Bradley et al. (1976) |
| | Low number of alveolar macrophages | Rothlein et al. (1981) |
| | Immature alveolar macrophages | Rothlein et al. (1981) |
| | Macrophages highly permissive to pseudorabies virus | Iglesias et al. (1989) |

B. STRESS-INDUCED IMMUNOSUPPRESSION

Many diseases of domestic food animals are known to involve an interaction of host exposure to stressful stimuli and viral and bacterial challenge. This concept seems to be especially relevant when one considers the etiology of respiratory and enteric diseases (Loan, 1984; Filion et al., 1984). The idea that stressed animals are more susceptible to disease generally relies on the assumption that alterations in immunocompetence have occurred (Table II). Indeed, some researchers have suggested that changes in immune function may be a useful indicator of stress in domestic food animals (Kelley, 1985; Siegel, 1985). Over the last decade several review articles have been written on the topic of stress and immunity in farm animals (Kelley, 1980, 1982, 1984, 1985,

TABLE II

EXAMPLES OF THE INFLUENCE OF STRESS ON SUSCEPTIBILITY TO INFECTIOUS DISEASE, HUMORAL
IMMUNITY, AND CELL-MEDIATED IMMUNITY IN DOMESTIC FOOD ANIMALS

| Stressor | Observation | Reference |
|---|---|---|
| Susceptibility to Infectious Disease | | |
| Cold | Increased susceptibility to TGE virus | Shimizu *et al.* (1978) |
| Draft and cold | Increased susceptibility to *H. pleuropneumoniae* | Verhagen *et al.* (1987) |
| Exertion (swimming) | Increased susceptibility to *Pasteurella multocida* when also exposed to ammonia | Neumann *et al.* (1987) |
| Transport | Increased susceptibility to infections | Staples and Haugse (1974); Mormede *et al.* (1982); Filion *et al.* (1984) |
| Regrouping | Increased susceptibility to Newcastle disease, hemorrhagic enteritis, Marek's disease | Gross and Colmano (1969); Gross (1972); Gross *et al.* (1988) |
| Humoral Immunity | | |
| Cold | Increased antibody production | Blecha and Kelley (1981); Kelley *et al.* (1981) |
| Transport | Decreased antibody production | Hartmann (1988) |
| Early weaning | Decreased antibody production | Blecha and Kelley (1981); Haye and Kornegay (1979) |
| Cell-Mediated Immunity | | |
| Cold or heat | Decreased DTH response | Regnier and Kelley (1981) |
| Transport | Decreased lymphocyte proliferation | Blecha *et al.* (1984); Murata *et al.* (1987) |
| Early weaning | Decreased lymphocyte proliferation | Blecha *et al.* (1983); Hennessy *et al.* (1987) |
| Restraint | Decreased DTH and thymus weight | Westly and Kelley (1984) |
|  | Decreased IL-2 production | Klemcke *et al.* (1987) |
| Exertion (treadmill) | Decreased lymphocyte proliferation | Blecha and Minocha (1983) |

1988; Albani-Vangili, 1985; Siegel, 1985; Blecha, 1988a; Griffin, 1989). If stress-induced changes in host immunity predisposes animals to disease, then methods of modulating the immune response in stressed animals should increase disease resistance (Blecha, 1988b).

When one attempts to intervene in an animal's response to a stressor, several different approaches can be envisioned (Fig. 1). Perhaps the best means of reducing the impact of stress on animal health is by providing a less stressful environment. However, deciding which environment or management condition is the least stressful is not a simple or easy task (Curtis *et al.*, 1989; McGlone and Hellman, 1988). Thus, several environments and management conditions have been evaluated for their influence on immune function (Blecha *et al.*, 1983; Blecha *et al.*, 1984, 1985, 1986; McGlone and Blecha, 1987; Minton *et al.*, 1988) and for their impact on the physiology of the animal (Dantzer and Mormede, 1983).

Another approach has been investigated as a method of reducing the influence of stress on susceptibility to disease: blocking the physiologic response to the stressor. The association between stress, neuroendo-
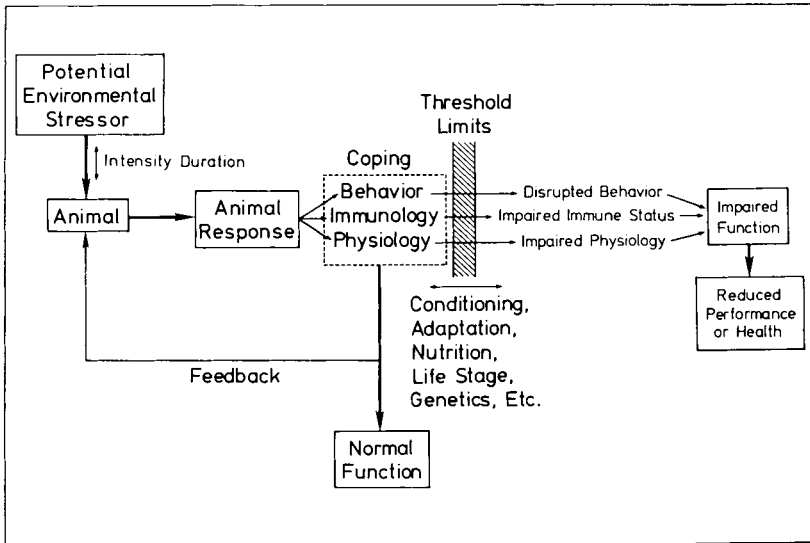


FIG. 1. Responses of animals to potential environmental stressors. Possibilities of improving performance and health in stressed animals can be envisioned by manipulating the behavior, immunology, or physiology of the animal. Taken from Hahn and Nienaber (1989), with permission.

crine responses, and alterations in immune function or disease susceptibility has been recognized for several years (Munck *et al.*, 1984; Kelley, 1988; Griffin, 1989). When increased concentrations of glucocorticoids have been associated with lower immune responses, administration of drugs that block the synthesis of corticosterone, such as metyrapone, resulted in an abrogation of the stress-induced immunosuppression (Blecha *et al.*, 1982). Recently, adrenal blocking chemicals (metyrapone and dichlorodiphenyldichlorethane) have been shown to increase the resistance of stressed chickens to viral and respiratory infections (Gross, 1989). Finally, when stress-induced immunosuppression has occurred, neurohormones, such as melatonin (Maestroni *et al.*, 1988), immunomodulating drugs (Hennessy *et al.*, 1987; Blecha, 1988b; Komori *et al.*, 1987), and cytokines (Conlon *et al.*, 1985) have been used to "up-regulate" or restore the immune response. It is likely that a combination of the approaches indicated above will provide the best means of reducing stress-induced disease problems in domestic food animals.

## C. PATHOGEN-INDUCED IMMUNOSUPPRESSION

Animals exposed to infectious disease often show depressed immune function. This is the case for several parasitic, bacterial, and viral infections. Pathogenic bacteria have been shown to affect immune responsiveness of infected animals. Thus, *Pasteurella hemolytica* or *Haemophilus pleuropneumoniae,* which both cause acute pneumonia in cattle and pigs, have been reported to exert toxic effects on lung macrophages and to alter macrophage phagocytic functions (Markham and Wilkie, 1980; Bendixen *et al.*, 1981). During bacteria-induced mastitis, suppressed responses in lymphocyte proliferation and neutrophil phagocytic functions have been reported (Nonnecke and Harp, 1988; Reddy *et al.*, 1989b). Immunosuppression of the host is also a frequent consequence of viral infections. Several examples of virus-related immunosuppression are well documented in domestic food animals (Table III), including viral diseases of great economic importance such as infectious bovine rhinotracheitis (bovine herpesvirus type-1) and pseudorabies, which cause severe pneumonia and death in cattle and pigs, respectively. As a consequence of virus-induced alteration of immune function, animals become very susceptible to secondary bacterial infections. The detrimental effects of these virus–bacteria synergistic interactions are of particular importance in the case of respiratory infections. Thus, following an initial viral multiplication in the lung, pathogenic bacteria proliferate, inducing the development of more se-

## TABLE III

### Examples of Virus-Related Immunosuppression in Domestic Food Animals

| Virus | Virus group | Host | Effects on the immune system | Reference |
|-------|-------------|------|------------------------------|-----------|
| Pseudorabies | Herpesvirus | Pig | Decreased lymphocyte proliferation and IL-2 production | Flaming et al. (1989) |
| Bovine herpesvirus-1 | Herpesvirus | Cattle | Decreased cell-mediated cytotoxicity; increased susceptibility to secondary infections; decreased IL-1 production; decreased lymphocyte proliferation | Babiuk et al. (1988) |
| | | | Decreased alveolar macrophage function | Blecha et al. (1987); Ghram et al. (1989); Carter et al. (1989); Brown and Ananaba (1988) |
| Parainfluenza-3 virus | Paramyxovirus | Cattle | Decreased lymphocyte proliferation | Ghram et al. (1989) |
| Rinderpest | Morbillivirus | Cattle | Lysis of infected lymphoid cells; decreased antibody response | Bielefeldt-Ohmann and Babiuk (1986) |
| Bovine leukemia virus | Retrovirus | Cattle | Decreased lymphocyte proliferation | Bielefeldt-Ohmann and Babiuk (1986) |
| Hog cholera virus | Pestivirus | Pig | Leukopenia; decreased lymphocyte proliferation; decreased antibody response | Charley et al. (1980); Van Oirschot (1983) |
| Bovine viral diarrhea | Pestivirus | Cattle | Decreased polymorphonuclear cell function | Roth and Kaberle (1983) |
| Transmissible gastroenteritis virus | Coronavirus | Pig | Lysis of infected alveolar macrophages | Laude et al. (1984) |

vere and acute lung lesions (Jakab, 1982; Babiuk *et al.*, 1988). If pathogen-induced immunosuppression can be moderated by immuno-modulating substances, then the prospects for domestic food animals to withstand disease should be increased. Stimulation of defense mechanisms, especially lung immune defenses, will likely require activation of local lymphoid cells such as alveolar macrophages (Charley, 1986). Targeting of immunomodulators to the critical organs will require special delivery systems, such as encapsulation in liposomes (Fogler *et al.*, 1980), which should be considered in the field of domestic food animal immunoenhancement.

## IV.  Summary

In the production of domestic food animals several situations exist where disease decreases production efficiency. Some of these diseases are exacerbated by a lowered or compromised immune response of the host. If immunomodulators can be used to augment immune function at critical periods during the production of food animals, such as the neonatal period, and prior to or during exposure to stressors or pathogenic organisms, then the economic loss caused by infectious disease should be reduced.

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