

Overview of the Immune Dynamics of the Digestive System

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Primary Audience: Poultry Producers, Flock Supervisors, Poultry Nutritionists, Researchers

SUMMARY

The digestive tract of the chicken is a major site of pathogen exposure. Although the bird has a multifaceted set of tools to prevent or resist infection, any activation of the immune system can divert nutrients away from production. Therefore, prevention of pathogenic exposure is preferred. However, it is unlikely that the bird can escape exposure to all pathogens during its life, thus the ability to respond to immunologic challenges is essential. The immune system of birds is similar to that of mammals in terms of structure and function, although some differences do exist, particularly in regulatory aspects. The innate immune system responds nonspecifically to foreign molecules and is essential for the induction of the specific (acquired) immune response. Cells of the innate immune system include macrophages, dendritic cells, heterophils, and natural killer cells. The acquired immune response involves recognition of a specific antigen and response by lymphocytes. Cytotoxic T lymphocytes are especially effective at inducing cells infected with intracellular pathogens to undergo apoptosis. Helper T lymphocytes increase the effectiveness of innate immune cells in combating extracellular pathogens and are also essential for activating B lymphocytes, which produce antibodies specific to the invading pathogen. All aspects of the immune system function together, although one aspect will often dominate, depending on the type and severity of the infection. This paper reviews the basics of avian immune function in general and discusses the immune system in the digestive tract in particular in birds. The consequences of activation of the immune system are presented.

Currently, growth-promoting antibiotics are not used in poultry in many countries; the North American industry may be moving in that direction as well, either through legislation or consumer pressure. Several nonantibiotic means of manipulating the immune system to prevent the health- and performance-suppressing effects of immune system activation are presented here.

Key words: immune function, poultry, innate immunity, acquired immunity, antibiotics

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DESCRIPTION OF PROBLEM

The wild jungle fowl and wild turkey, the ancestral species of modern commercial broilers and turkeys, respectively, lived under vastly different conditions than those in which modern

production birds are currently housed. The wild birds had an extensive environment with freedom to move within their range and would have been exposed to any predators, pathogens, and parasites within that range. In addition birds foraged for food, the availability and nutrient levels

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of which would vary, depending on season, growing conditions, and geography. In contrast, modern production of poultry is practiced under conditions very different than that of the environment in which the ancestral wild jungle fowl or turkeys would find themselves.

To efficiently and economically produce the large volume of meat and eggs required to meet consumer demands, chickens and turkeys are most commonly raised intensively at high population densities. The birds have undergone intensive selection for particular traits, such as meat or egg production. Diets are formulated to meet the needs of the bird based on production type, age, and level of production with consistency in ingredient and nutrient composition from day to day. Management practices such as biosecurity, vaccination, and the use of growth-promoting antibiotics have been implemented to not only reduce the risk and consequences of disease but also to increase productivity.

The most important role of the immune system of a wild bird is to ensure survival of the bird. Therefore, consequences of an immune response, including decreased growth rate, cessation of reproductive activity, and metabolic inefficiencies are all acceptable costs associated with overcoming a disease challenge. In the context of modern, economically efficient poultry production, success is defined not only in terms of survival but also in terms of maintenance of high rates of growth or egg production.

ROLE OF THE GUT

In the most simple of descriptions, a chicken can be thought of as a tube with the lumen of the intestinal tract being outside of the bird. Material ingested by a bird contains nutrients, nonnutrients, and beneficial and potentially harmful organisms and material. The lumen of the digestive tract of poultry normally contains feed and its constituents, resident and transient microbial populations, endogenous nutrients, and secretions from the gastrointestinal tract (GIT) and accessory organs such as the liver, gall bladder, and pancreas. The primary purpose of the GIT is to digest and absorb nutrients from the ingested feed. The GIT must selectively allow the nutrients to cross the intestinal wall into the bird while preventing the deleterious components of the diet from crossing the intesti-

nal barrier. In addition to simply preventing access to the bird by blocking entrance, immune tissues and cells within the gut actively respond to microbial challenges. Although generally microscopic in size, pathogenic organisms are too large to enter the enterocytes and must invade the tissues of the bird to colonize and thrive.

OVERVIEW OF IMMUNE RESPONSES IN POULTRY

The basic functions of any immune system are the distinction between self and nonself and the appropriate response to identification of each. Birds, like other animals, have nonspecific and specific immune mechanisms to respond to potential infectious threats from bacteria, viruses, parasites, and other antigenic material. The 2 arms of the immune response are innate (nonspecific) and acquired (specific) immunity. These 2 types of responses are usually coordinated; pathogenic challenges are initially processed by the innate system, and, if necessary, the acquired response is subsequently activated. The general mechanisms of immunity are identical in chickens, mammals, and most other vertebrates [1, 2].

Innate and adaptive immunity are essential for host survival and health. The innate response is generalized to broad classes of foreign (non-self) immunological challenges, including pathogens. The innate response has systemic effects on host physiology, and activation of this system can divert substantial amounts of nutrients away from growth [3]. Acquired immunity is targeted at specific antigens, and response to a particular pathogen involves a very limited subset of lymphocytes. The acquired response is exquisitely specific and targeted; activation of this type of immune response requires very little in the way of nutrients and causes very little change in metabolism of the bird [4, 5]. For the host to be fully protected, these 2 aspects of the immune system must work in concert. Therefore, despite the negative effect of an innate immune response on growth and productivity of poultry, attempts to improve performance through manipulation of innate immune function must be done with this in mind. The most effective means of reducing the effect of the immune system on bird performance is to reduce exposure to foreign antigens.

Innate Immunity

Innate immunity involves the inherent non-specific mechanisms a bird has to resist disease. The innate immune system is present and functional at hatch, although development continues to occur during the first week of life [6]. This arm of the immune system is the first line of defense against invading pathogens. The first aspect of innate immunity is the exclusion of pathogens by barriers to entry: the skin and mucosal surfaces [7]. Any foreign organism or antigen that crosses these barriers is then encountered by intraepithelial leukocytes, including macrophages, dendritic cells, heterophils, natural killer (NK) cells, and T lymphocytes [7, 8, 9]. All but the lymphocytes are generally considered to be cells of innate immunity. Innate immune cells have several functions, including the recognition and control of invading pathogens as a first line of immunological response as well as antigen presentation and subsequent activation of the mechanisms of acquired immunity.

Macrophages are among the first cells encountered by an invading pathogen. Macrophages kill extracellular pathogens through endocytosis and subsequent formation of enzyme- and toxin-containing lysosomes [10]. These phagocytic cells secrete cytokines that attract other immune cells to the site of infection. Blood-derived monocytes begin to infiltrate the infected tissue and differentiate into macrophages [11]. Macrophages are also capable of antigen presentation to T and B cells [11].

Dendritic cells are present in most tissues in the immature form but are the most potent antigen-presenting cells for activation of naïve T cells [12]. In this state, they are capable of capturing and processing antigen on their cell surfaces. At this point, they mature and migrate to lymphoid tissues, where they present antigen to and activate antigen-specific T cells [9]. Lymphoid nodules exist in various tissues of the bird and are formed *de novo* at the site of an infection. In these lymphoid nodules, the T cells encounter the cell-bound antigen, and induction of the acquired immune response begins. Mature dendritic cells can also interact with macrophages to stimulate cytokine release and with B cells to induce antibody formation [9]. Full T-cell activation and clonal expansion can take 4

to 5 d. During this time, the innate immune system continues to function to limit the growth of the pathogen and receives increasing assistance from the acquired immune response.

Heterophils are the functional equivalent of mammalian neutrophils [13] and are the primary cell type recruited to the site of infection during an inflammatory response [13]. Mast cells mediate vasodilation and increase vascular permeability, allowing heterophils and other cells to infiltrate the inflamed tissues [14]. Heterophils are phagocytic [15, 16]; exposure to chemokines and cytokines increases their phagocytic and bacteriocidal activities [17, 18]. Cytokines and chemokines released by heterophils attract other innate and acquired immune cells to the site of infection. Avian heterophils kill pathogens primarily through nonoxidative means [13], relying instead on oxygen-independent mechanisms [16]. Granules contained within the heterophil have cationic bacteriocidal peptides, lysozyme, acid peptidase, acid hydrolases, cathepsin, and α -glucosidase [13]. Release of granules from granulocytic cells such as heterophils can lead directly to tissue damage [19, 20].

Natural killer cells are cytotoxic to tumor cells and cells infected by viruses or other intracellular pathogens [21, 22]. The NK cells do not have a T-cell receptor that allows them to recognize specific antigen and are thus considered to be innate immune cells [1].

The innate response is not specific in that the immune cells recognize not a specific antigen but, rather, generalized conserved molecules common across many pathogens through pathogen-associated molecular patterns [4] and other receptor-mediated methods [11]. This nonspecific recognition of a wide array of foreign invaders allows the innate immune system to respond to challenges in a much shorter time than the acquired immune response [22]. Therefore, the innate immune response is essential in the days or weeks following pathogen exposure during which the acquired immune response gradually takes on a greater role in host defense.

The innate immune system will respond to different foreign pathogens in much the same manner, regardless of the species of invading organism. The response may vary in severity and duration, but once the challenge has been defeated, the response diminishes, and no immu-

nologic memory is retained by this branch of the immune system.

Acquired Immunity

As its name suggests, acquired, or adaptive, immunity must be developed by a bird. To respond to a unique, novel pathogen, a bird must first activate the mechanisms responsible for dealing with that particular invader. Acquired immunity is specific and heterogeneous and has memory. Thus, a specific pathogen will be recognized by the immune system in a particular way; different pathogens elicit different responses, and reexposure to the pathogen will often result in a more rapid, effective response than was elicited at the first exposure. Acquired immunity involves 2 types of responses: cell mediated and humoral [23]. In cell-mediated immunity, cells infected with a foreign pathogen are destroyed via interaction between the infected cell and an effector cell such as an activated T cell [23]. Humoral immunity is mediated by antibodies produced by B cells in response to an antigenic challenge. The 2 types of lymphocytes are phenotypically different; T cells express T-cell receptor complex on their surface, whereas B cells express immunoglobulin on their surface [24]. The regulation and effectiveness of avian adaptive immunity is comparable to that in mammals [1].

Major Histocompatibility Complex

The MHC is a set of molecules displayed on cell surfaces that are responsible for presentation of antigenic peptides such that they are recognizable by antigen-specific T-cell receptors. The antigens presented in this way are either self (i.e., peptides derived from normal, healthy cells of the host's body) or nonself (foreign antigens originating from bacteria or viruses). Under normal circumstances, T cells will ignore cells presenting only self-antigen and react to cells presenting foreign antigen. Chicken MHC structure and function is similar to that of most vertebrates [25]. There are 2 types of MHC molecules, class I and class II MHC that allow surveillance of the body for foreign antigen [25]. Class I MHC molecules are present on almost all cells of the body, whereas class II MHC molecules are present only on the surface of

antigen-presenting cells [26]. Class II MHC is involved in presentation of antigen from antigen-presenting cells to T cells but also from helper T cells to B cells [27, 28].

Antigen Specificity

The number of T cells that will recognize and respond to a given antigen is extremely small. However, once the antigen is recognized, the specific T cell is activated. Clonal expansion results in a vast increase in the number of the appropriate antigen-specific cells; this newly expanded population then begins to mature.

Cell-Mediated Immunity

The cell-mediated aspect of the acquired immune system is directed by T lymphocytes (T cells). Cytotoxic T lymphocytes (**CTL**) recognize infected cells and release lymphotoxins that cause apoptosis. T-helper lymphocytes (**T_H cells**) direct the immune response, secreting lymphokines that stimulate CTL and B cells to grow and divide; attract heterophils; and potentiate engulfment and destruction of microbes by macrophages. Suppressor T cells inhibit the activity and production of cytotoxic T cells after successful elimination of the pathogen, thus preventing excess damage to the host's own tissues. Memory T cells will recognize and respond rapidly the next time a particular pathogen is recognized [1].

CTL. Cytotoxic T lymphocytes are most effective against intracellular pathogens such as viruses. The virus uses the host cell mechanisms to replicate itself; the killing of infected cells will stop viral replication. The CTL express the cell surface protein CD8 on their surface [1]. CD8 is used as a coreceptor by CTL to identify target cells via MHC class I-dependent antigen presentation [12]. Class I MHC molecules generally bind short peptides proteolytically derived from intracellular proteins, including host proteins. Only then can MHC-bound antigen be recognized by cytotoxic T cells. If the antigens presented are self, the cell is not attacked. If the antigen presented is from intracellular pathogens, such as viruses and some bacteria that use the cell's synthetic machinery, cytotoxic T cells attack the cell. Without MHC class I molecules, the antigen would remain hidden from the im-

immune system [25]. All nucleated cells express MHC class I, and therefore no specialized antigen-presenting cell is necessary. This means that any infected nucleated cell in the body can be targeted by CTL [1].

An activated CTL specific for an antigen identifies the infected cell and induces apoptosis, a process also known as programmed cell death. Infected target cells are specifically induced to undergo apoptosis, in which the cell is orderly dismantled with subsequent removal of the cell components. Induction of apoptosis prevents intracellular components from leaking into the surrounding tissue, which could cause inflammation and damage to other host cells [10]. The process of avian CTL-mediated apoptosis of target cells appears to be similar to that of mammals [1].

The NK cells use killing mechanisms similar to that of CTL and are especially important prior to activation of CTL. NK cells appear to target cells that lack MHC class I expression [1]. Many intracellular pathogens downregulate cell expression of MHC I to avoid detection by CTL; in this case, the importance of NK activity increases. The killing of infected cells is targeted, and neighboring cells that are uninfected are not acted upon by CTL or NK.

T_H Cells. Adaptive immunity is dependent on regulation by T_H cells [29]. T_H cells express the cell surface protein CD4 on their surface and are involved in defense against extracellular pathogens. CD4 acts as a coreceptor to allow T_H cells to recognize an antigen presented in the context of MHC class II molecules [1, 25]. Normally, antigen-presenting cells for T_H cells are limited to dendritic cells, macrophages, epithelial cells, M cells in the Peyer's patches, and B cells [1, 30]. Whereas CTL can interact with almost any cell in the body, T_H cells necessarily interact with specific cells of the immune system.

When an antigen-class II MHC complex is recognized by antigen-specific T-helper cells, antibodies, proinflammatory cytokines, or both are produced [25]. The pattern of cytokines released by antigen-presenting cells directs the type of adaptive immune response that is mounted. In mammals, antigen-presenting cells produce cytokines that direct naïve T_H cells (T_H0) to differentiate into T_H1 or T_H2 cells [1].

Interleukin-12, interleukin-18, and interferon- γ favor differentiation to T_H1 and, consequently, a cell-mediated response. Interleukins-1, -4, -6, and -10 favor T_H2 differentiation and, consequently, an antibody-mediated response [1].

The differentiated T_H cells also express cytokines following activation. The cytokines produced by mammalian T_H1 cells are typically interferon- γ , tumor necrosis factor- α , and interleukin-2. A T_H1 response increases the effectiveness of targeting and killing of pathogens by macrophages [1] and heterophils [17, 18]. Innate immune cells, macrophages, and heterophils are also part of the cell-mediated response in that they can be activated by T_H1 cells [1]. Likewise, NK cells are innate immune cells but can also be considered as a part of the cell-mediated response because their proliferation and cytotoxic function can be increased by exposure to T_H1 cytokines [10]. Some organisms are capable of infecting macrophages and preventing some of the steps involved in pathogen killing. In such cases, T_H1 cells can release cytokines that increase production of reactive oxygen intermediates, reactive nitrogen intermediates, and oxygen-independent antimicrobial factors by macrophages to eliminate the infection [10].

A T_H2 response activates B cells in the humoral response and is an essential step in the production of antibodies. T_H2 cells typically produce interleukin-4, interleukin-5, transforming growth factor- β , and interleukin-10 [1].

The pattern of cytokine secretion associated with a T_H1 response minimizes the secretion of T_H2-associated cytokines and vice versa [1]. Therefore, one type of T_H response will tend to predominate while the other is minimized. The specifics of this regulation of events are less clear in birds, but the end result seems to be the same as in mammals [1]. The functional capabilities of avian T_H1 cytokines are very close to that of mammals, even though there seem to be substantial structural differences [31]. T_H2 cytokines in avian species are not well defined [1].

Immature T_H cells are activated, then proliferate, and then differentiate into effector cells or memory cells [1]. After a successful acquired immune response, the antigen is no longer present, and most of the antigen-specific clone cells begin to die off. However, some T cells remain

as memory cells and are present throughout the animal's life at levels 100- to 1,000-fold higher than the same antigen-specific naïve cells were initially [12].

A full T_H response can take between 4 to 7 d to reach protective levels of activity. During that time, the innate immune system continues to fight off the pathogen. As discussed previously, the innate response often has systemic effects on metabolism, effects that decrease the rate and efficiency of production in poultry. Although the innate immune system is essential in antigen processing and host defense, inappropriate or excessive activation of innate responses can have a negative effect on the health of the bird [19, 20] and, therefore, the economics of poultry production.

Humoral Immunity

The second type of adaptive response is humoral immunity, the response mediated by antibodies carried in the blood. B lymphocytes are the effector cells of the humoral response. When a naïve B cell encounters its specific antigen in the blood, a surface immunoglobulin acts as a receptor to allow the B cell and antigen to bind [12]. Before it can become activated, the B cell must then bind to an antigen-specific, activated T_H2 cell. The T_H2 cell releases cytokines, which in turn primes the B cell to undergo clonal selection, an asexual mitotic reproduction of the particular cell. Most of the clones become plasma cells, which after an initial lag are capable of producing immense numbers of antibody molecules. The antibodies (immunoglobulins or Ig) inactivate antigens by several methods [1, 12] that include the following:

Complement Fixation. Proteins from the plasma bind to the antigen surface and cause cell lysis.

Opsonization. Antigens are coated with molecules such as immunoglobulins or complement proteins, which increases the effectiveness of phagocytosis via receptor-mediated endocytosis.

Neutralization. The binding sites of the antigen are occupied, preventing the attachment of the antigen to a host cell. If attachment cannot take place, infection will not take place.

Agglutination. Antibodies bind to more than one antigen molecule (i.e., on multiple pathogenic organisms). The pathogenic cells clump together and are not able to infect the host.

Precipitation. Attachment of the antibody to the pathogen causes it to become insoluble and come out of solution.

The life span of a plasma cell is about 4 or 5 d. The other B-cell clones become memory cells and remain in the body for long periods, allowing a rapid, specific antibody response the next time the pathogen is encountered.

As with naïve T lymphocytes, there are very few B cells that will recognize and react with a particular pathogen. The vast range of specificities in B-cell antigen recognition is the result of immunoglobulin gene rearrangement in proB cells [32].

Once the antigen is no longer present in the body, the antigen-specific B cells begin to die off. However, some of the B cells become long-lived memory cells, present at 10- to 100-fold levels higher than initially, but also more potent in antibody-secreting capacity than unprimed B cells specific for the same antigen [12]. Unlike most mammals, this B-cell development early in a bird's life takes place in the bursa of Fabricius [33] rather than in the bone marrow [32].

PROGRESSION OF THE IMMUNE RESPONSE

When a novel pathogen is encountered for the first time, it must first be recognized by the bird as nonself. In the recognition phase, innate immune cells respond by engulfing and killing of the pathogenic invader and processing of antigen. The activation phase involves the recruitment, proliferation, and activation of lymphocytes, and the effector phase results in the elimination of the pathogen using antigen-specific mechanisms. The extent of involvement of the innate, cell-mediated, and humoral aspects of the immune system will vary depending on the type of pathogen, the severity of the challenge, the overall health and nutritional status of the bird, and many other factors. In many cases, the innate immune response is sufficient to resolve the challenge. In that case, the acquired response is not activated, and there will be no immunological memory of the pathogen.

Once the pathogen has been cleared from the bird, the antigen is no longer present to stimulate the antigen-specific T and B lymphocytes. The clones begin to die off, although a few memory cells remain in circulation. The memory cells allow a more rapid response the next time that the antigen is encountered.

If a pathogen has been encountered previously by a bird and the innate immune system has been successful in resolving the challenge, no immunological memory will exist, and the second exposure is handled in the same manner as exposure to a novel pathogen. If, however, the acquired immune system has previously responded to the pathogen, memory T and B cells may remain. Memory of particular pathogens may decrease with time at a rate that varies with pathogen type, severity of the challenge, and other factors. However, as long as memory cells remain, the next time the pathogen is encountered, a much more rapid response by the acquired immune system can be produced. The innate immune system will still be required for antigen processing and presentation as well as the initial defense of the bird. The transition from innate to acquired immune system dominance, however, will be much more rapid.

GIT IMMUNITY

One of the main routes of access of pathogens to the bird is via the mucosal surfaces of the gut, and the gut has an essential role in protection from disease [30, 34, 35, 36]. Therefore, an appreciation for the role of the GI tract, distribution and function of the immune system within the gut, and means of manipulating those interactions to the benefit of birds and producers is essential for continued safe and efficient production of poultry products for human consumption.

Epithelial and Mucosal Barrier

The epithelial surface of an organism is an essential component of the immune system and prevents access to the host by foreign molecules, including pathogens [7]. The mucosal surface, which includes the GIT, is the largest surface of interaction between the bird and the outside world. The intestinal tract is necessarily exposed to a wide variety of foreign molecules and mi-

crobes. The mucosal barrier and gut-associated lymphoid tissue (**GALT**) are essential for protection from invasion by pathogenic organisms. Pathogenic organisms are trapped in the mucosa where they are inactivated by secreted products, such as secretory IgA [30, 36], lysozyme, and other antimicrobial compounds [37]. The inactivated microbes are then unable to colonize and proliferate and are passed out of the digestive tract.

Symbiotic Bacteria

Although not part of the bird, the normal microflora of the gut is an essential component of host protection. These microbes occupy ecological niches within the digestive tract [38] and prevent colonization by pathogenic organisms [39, 40, 41]. Although the normal gut microflora also has antigens recognized by the bird as being foreign, their presence does not normally elicit an overt immunological response [34, 36]. This is termed immunological tolerance [12].

GALT

The GALT is collectively one of the largest secondary immunological organs in the body. The GALT comprises immune tissues and cells found within the epithelial layer and lamina propria of the digestive tract. Cells involved in antigen presentation, immunoregulation, and effector function are present in GALT [30]. The immune cells can occur as scattered individual cells responsible for surveillance of foreign material that may enter a bird's tissues or as discrete lymphoid aggregates [2, 42] where antigen presentation to the appropriate effector cell is more likely due to the concentrated populations of immune cells. The GALT lymphoid aggregates in birds include the esophageal tonsil, Peyer's patches, cecal tonsils, the bursa of Fabricius, and localized lymphoid follicles that form near a site of infection [7, 30, 36, 42]. Most immune cell types are present in the lamina propria of the gut, including macrophages, granulocytes, plasma cells, effector T lymphocytes, and memory lymphocytes [42]. The distal portion of the GIT is exposed to greater microbial loads than other portions of the digestive tract and as such has a greater GALT presence than more proximal regions [43].

IMPACT OF IMMUNE SYSTEM ACTIVATION

As discussed previously, the innate immune system is the first line of defense against invading pathogens. Passive components of this system, such as the skin and mucosal surfaces, keep antigens outside of the bird, prevent exposure of the active components of the immune system, and thus prevent the cascade of events of an immune response from occurring. Biosecurity and sanitation programs are an integral part of minimizing the effects of immune system activation in poultry production. The metabolic costs of barrier defenses are largely due to maintenance of that barrier. However, when a pathogen gains access to the host by evading the protective barrier, an active response is required. The first line of defense in this case is the innate immune system. Systemic metabolic changes occur that nonspecifically inhibit colonization by and growth of pathogens within the bird. Exposure to microbial challenges decreases growth rate [44, 45, 46, 47] and accretion of skeletal muscle [48]. An infectious challenge can decrease feed intake of broilers by 70% [49]. The metabolic changes also include fever, metabolic inefficiencies, skeletal muscle catabolism, and acute phase protein synthesis [4, 49]. All of these responses result in the diversion of nutrients and energy away from growth. The acquired immune response is a targeted response and requires very little in the way of nutrient or metabolic cost [4, 5, 50, 51]. The innate system is a necessary but metabolically and economically costly means of host protection.

The innate immune response is a blunt instrument. Many of the means of resisting infection are systemic and are meant to create an inhospitable environment and limit microbial growth [4]. A fever limits the growth of bacteria by moving body temperature above the optimal range for many pathogens. A decrease in appetite and, therefore, feed intake prevents the further ingestion of foodborne pathogens. At the same time, body tissues are broken down and fat reserves are used for energy, and skeletal muscle is broken down to provide amino acids to the liver for acute-phase protein synthesis. The acute-phase proteins are involved in an array of mechanisms meant to limit microbial growth.

Nutrients, such as many minerals and vitamins, are removed from circulation and stored in tissues, limiting their availability to be used by invading microbes. These effects of innate immune function are systemic, and conditions created are not only limiting to the growth of pathogens but often limit growth or egg production of the bird as well.

Because birds will be exposed to foreign material and antigens in poultry houses, a greater understanding of how the immune system functions and is regulated is essential. To reduce the production-suppressing effects of pathogen exposure, means of manipulating the immune system must be investigated. The goals of such research should include increasing the efficiency of pathogen elimination by the innate system while minimizing the systemic effects of activation. Achieving a rapid transition from an innate response to the more efficient, targeted response of the acquired immune system may reduce the decrease in production in commercial poultry caused by immune system activation.

CHALLENGES AND OPPORTUNITIES

Antibiotic Growth Promoters

The poultry industry has used growth-promoting antibiotics to great advantage in terms of bird performance since the 1950s [52]. Although the mode of action is not entirely understood, and different antibiotics have different mechanisms, one of the primary effects of subtherapeutic levels of antibiotics seems to be a reduced activation of the inflammatory response [53, 54]. Subsequently, more nutrients are available for growth rather than for the systemic metabolic changes associated with inflammation [4, 55].

In many poultry production areas of the world, growth-promoting antibiotics have been removed partially or entirely from feed, either through legislative action or through companies voluntarily eliminating antibiotic growth promoters to address consumer concerns [52, 56, 57]. It is not unreasonable to assume that North American and many European countries will drastically change the use of antibiotic growth promoters in the near future.

The impending loss of, or change in, the usage of antibiotic growth promoters has re-

sulted in intensified efforts to find new ways of reducing the exposure of pathogenic microbes to the bird or modulating the way in which the bird responds to such challenges. The mechanisms discussed below are by no means an exhaustive list of immunomodulatory approaches. Rather, these are a few of the approaches that may become part of the standard management practices used by the poultry industry to continue to produce economical, safe, and wholesome food.

Genetic Selection for Disease Resistance

Genetic selection for altered immune response has been used effectively by the poultry industry in the past, perhaps most notably in certain MHC haplotypes conferring resistance to Marek's disease in chickens [58, 59]. Continued selection of poultry for traits in addition to growth and efficiency will likely play a major role in the advancement of health and productivity in poultry [60]. However, it should be noted that in general, activation of the immune response has a negative effect on growth rate, and selection of lines of chickens for increased specific responses results in lines of birds with poorer growth rates [61, 62, 63]. Because of this, genetic improvements in the ability of birds to resist various diseases may come at a cost of reduced growth rate or slowed advances in increases in growth rate. Because different pathogens elicit different types of immune responses, selection for resistance to a particular disease may make the bird more susceptible to other diseases. A greater understanding of immune system regulation is necessary for application to poultry breeding programs in the future.

Exogenous Enzymes

Many parts of the world use wheat or barley in poultry diets. Although more readily available than corn in many locations, these cereals may contain high levels of indigestible, soluble non-starch polysaccharides (NSP). High levels of NSP in the diet cause increased water intake and litter moisture; increased digesta viscosity, which decreases in mixing (thus reducing enzyme-substrate interactions and absorption of released nutrients); and decreased production [64, 65, 66, 67, 68, 69]. Of increasing research

interest is the effect that NSP have in altering intestinal microbial populations of animals [54, 70]. The use of wheat in poultry diets may favor pathogenic bacteria such as *Escherichia coli*, *Salmonella*, clostridia, and *Campylobacter* [71, 72, 73, 74], possibly due to the creation of a more anaerobic environment. The decrease in mixing associated with increased digesta viscosity reduces enzyme-substrate interactions, leaving more feed undigested in the GIT. Additionally, substrate liberated following enzymatic cleavage will be less likely to migrate to the absorptive surface of the intestine, making it more available for use by intestinal microbes, including pathogens, and less available for absorption by the bird. Recent research [75] has shown that, like wheat, corn source and quality can affect microbial populations in poultry; these effects can be modulated with exogenous dietary enzymes.

Probiotics and Prebiotics

Probiotics are live cultures of bacteria that are fed to poultry to hasten the development of stable, normal gut microflora. Most often, this involves feeding the cultures to poultry beginning early in the life of the bird to rapidly establish a protective layer of beneficial bacteria lining the GIT. The chick hatches with a sterile digestive tract, and microbial colonization begins almost immediately. The profile of microbial species present in the GIT changes as the microflora develops. Some species appear and disappear [76] while the population becomes more complex in its makeup. Microbial populations are in a state of flux during the early part of a bird's life [77]. The sooner a healthy, stable gut microflora occupies all of the ecological niches in the gut, the less likely colonization by pathogens becomes. In order for pathogenic bacteria to proliferate in the gut, they must have attachment sites. If no attachment sites are available, the microbes are swept along with the digesta and removed from the bird via defecation.

Because the microbial community of the intestinal tract is exceedingly complex, it is the most complex probiotic preparations that are most protective for the bird [78]. Many countries will not approve undefined probiotic cultures on the basis that, without knowing exactly which microbial species are present in the preparation,

pathogens may be fed inadvertently to the birds, thereby increasing rather than reducing colonization by pathogens. Because of the difficulty of creating and maintaining complex defined culture probiotics, most commercially available preparations contain one or a few species of bacteria, generally lactobacilli. These preparations are usually far less effective in conferring protection against pathogen colonization than more complex or undefined cultures [78].

Similar to the concept of probiotics is the concept of prebiotics. For bacteria to thrive in an ecological niche, environmental variables must be suitable (e.g., temperature, pH, presence or absence of O₂), and the bacteria must have access to sufficient quantity and quality of nutrients to allow them to thrive. Prebiotics are molecules included in the diet that are intended to feed the beneficial microflora of the gut [79]. When probiotics and prebiotics are provided together in the diet, the introduction and maintenance of beneficial bacterial populations may be accomplished [80, 81].

Nutritional Immunomodulators

The cells of the immune system require nourishment to function properly. However, many dietary components appear to have effects on the immune system beyond simply providing nutrients. Although the list of such nutrients is extensive [82, 83, 84], one example is given below.

n-3 Polyunsaturated Fatty Acids

The n-3 polyunsaturated fatty acids can be used in poultry rations to alter the inflammatory response and reduce the systemic effects of pathogenic challenges. Birds fed diets containing relatively low amounts of fish oil (0.5 to 2%) had reduced interleukin-1 production by macrophages and improved growth rates when challenged with bacterial lipopolysaccharide [44]. Similar levels of fish oil has resulted in increased inflammatory cell infiltration to the site of an experimental coccidiosis challenge but a decrease in the systemic effects of infection [46]. This latter result suggests that dietary fish oil may be a means of somewhat uncoupling the

localized immune response necessary for dealing with a pathogen from the systemic, production-suppressing effects of inflammation.

Exogenous Dietary Antibodies

For GIT pathogens to infect a bird, they must be able to get through the protective barrier of the mucosal surface. This can occur when the gut lining is damaged (e.g., in the case of secondary necrotic enteritis following tissue damage due to coccidiosis [85]) or through active invasion by the pathogen. Exogenous antibodies can be fed to poultry to bind up antigens present on the surface of pathogens, causing the microbes to clump together and prevent colonization of the GIT. As relatively large molecules, the antibodies are not absorbed from the intestine [86]; their effect is entirely within the gut.

Antibodies may be produced in a number of ways, but one of the most promising is through vaccination of laying hens with antigens of particular relevance to poultry production. The hens mount an antibody response and pass on maternal antibodies into the eggs. The eggs are processed commercially, the antibodies are removed and purified, and the remainder can be used in the liquid egg market. Thus, not only are the hens producing food but also a high-value by-product [87]. The purified antibodies can be added to the feed of other birds to offer protection against the specific pathogen(s) against which the hen was immunized [86, 87]. More recent research has focused on the production of antibodies to specific molecules involved in inflammation, such as neuropeptides and phospholipase A₂ [87].

The use of exogenous antibodies is advantageous in that the product is natural, and development of microbial resistance is unlikely. However, the maternal antibodies produced will be specific for the antigens to which the hens have been exposed. Inclusion of exogenous antibodies will offer no protection to novel, unrelated pathogens. From a practical standpoint, exogenous antibodies may be more applicable when specific pathogens are expected to be encountered or to reduce the risk of specific foodborne human pathogens.

CONCLUSIONS AND APPLICATIONS

1. The immune system has 2 separate aspects, innate and acquired immunity, that function together to protect the bird from exposure to foreign material and microbes.
 2. In general, activation of the immune system decreases performance of modern poultry. To maximize production efficiency, the immune system should be kept in surveillance mode unless activation is required. Once activated, a rapid resolution or transition from innate to acquired immune responses is desirable to minimize the loss of productivity.
 3. The GIT is a major site of interaction between the bird and foreign material, some of which may be pathogenic. The GALT has a major role in minimizing access of pathogens to the bird.
 4. The GALT has aspects of the innate (nonspecific) and acquired (specific) arms of the immune system, offering protection against novel invaders and repeat exposures.
 5. Manipulation of the immune system, including at the gut level, may allow nonantibiotic means of ensuring maintenance of and even continued improvements in the performance and safety of poultry as food products.
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