Impact of nutrition on the innate immune response to infection in poultry

M. H. Kogut

Southern Plains Agricultural Research Center, USDA-Agricultural Research Service, College Station, TX 77845

Primary Audience: Poultry Producers, Poultry Nutritionists, Researchers

SUMMARY

Viral and bacterial diseases remain a threat to the poultry industry and countermeasures to prevent and control them are needed due to production losses. With the continued threat of exotic and emerging diseases and concern over the use of antibiotics in animal production, there is a serious and urgent need to find safe and practical alternatives to prevent or control pathogens. Identification of new tools for the design of new immunological interventions or therapeutic antimicrobials to reduce microbial pathogens in poultry is now required more than ever. Immunological interventions to reduce microbial pathogens in poultry would be of great value to the poultry industry and to the consumer. We have been advocating boosting immunity and encouraging the host to utilize its innate immune system to control and clear infections. Our research has addressed the use of innate immune mechanisms and components to develop new immune modulators (prophylactic and therapeutic) and the characterization and production of antimicrobial peptides as potential immune modulators in poultry. Dietary bioactive food components that interact with the immune response have considerable potential to reduce susceptibility to infectious diseases. With this premise, this paper asks and answers a series of pertinent questions on the utilization of avian immunity for increasing resistance to a variety of potential pathogens problematic in today’s commercial poultry industry. Using experimental data to provide answers to these questions, we hope to stimulate a dialog between avian immunologists and nutritionists that results in coordinating and integrating their expertise into specific practical solutions that will benefit the industry and improve the well-being of commercial poultry.

Key words: innate immunity, nutrition, poultry, immune function

DESCRIPTION OF PROBLEM

Infectious diseases remain a threat to the poultry industry and countermeasures to prevent and control them are needed. With the continued threat of exotic and emerging diseases and concern over the use of antibiotics in animal production, there is a need to identify and implement practical alternatives to prevent infectious diseases. New immunological interventions and therapeutic antimicrobials to reduce microbial
pathogens are 2 methods currently being investigated worldwide. Our laboratory’s research has focused on targeting immunomodulatory and antimicrobial compounds directed at critical control points of the host-pathogen interface.

Dietary bioactive food components that interact with the immune response have considerable potential to reduce susceptibility to infectious diseases. Major classes of macronutrients provide numerous examples, including amino acids such as arginine or threonine [1, 2], lipids such as the n-3 polyunsaturated fatty acids, or novel carbohydrates such as various sources of β-glucans [3–7]. Vitamins such as D and E are commonly used as antioxidants, whereas zinc and selenium are minerals with a wide spectrum of effects on the immune system [8–11]. There is accumulating evidence for prevention of infectious diseases by probiotics and prebiotics, and these may also affect the immune response [5].

However, that being said, this paper is not a review of nutritional effect on the avian immune system. There are several recent reviews that are excellent and should be read [3–6]. I have been asked to approach the interaction between nutrition and immunology from an immunologist’s perspective and try to answer specific questions that could help nutritionists not only understand the avian immune response, but also to use this understanding in directing nutritional immunology research. Now it must be stated that I am not a nutritionist by any definition. However, that is not to say that our work has not delved into the use of feed additives and their interactions with the avian immune system. Therefore, for the purpose of this manuscript, I am going to elaborate on some of our research tactics that provide a reasonable basis for an applied approach that can be used by the industry, if not immediately, then certainly in the future. Now to be sure, nutrition alone cannot, and will not, be the magic bullet to provide for bird health; this requires a coordinated, integrated approach that combines physiology, metabolism, genetics, and immunology.

To provide the most complete picture, this paper will concentrate on asking a series of pertinent questions on avian immunology and using data that provide answers that can form the framework for formulation of potential diets that can provide nutritive benefits and also increase resistance to a variety of potential pathogens in poultry. Specifically, the questions that we address here include: (a) what is an optimal avian innate response, (b) can the innate response be modulated, (c) do we want sustained modulation of the innate response, and, if not, (d) when is best time for modulation using dietary components as immune modulators, and (e) can dietary intervention strategies be tailored for optimal bird health (i.e., do nutrigenomics have a place in poultry health)?

WHAT IS AN OPTIMAL IMMUNE RESPONSE?

The hallmark of an immune system is the diverse and variable responsiveness to potential pathogens. It is this diversity and variability that allows the immune response to defend against multiple types of infectious agents and to eliminate those agents from the host, especially in genetically homogenous populations of today’s commercial poultry industry. However, we must keep in mind that the downside to variability is that infections with less virulent pathogens can still affect productivity. Interestingly, selection of today’s modern chickens for growth and egg production has resulted in a diminished inflammatory response [12], but selection for more hearty immune responses results in diminished growth and egg production [13, 14]. So, with this dichotomy in mind, what do we consider an optimal immune response in poultry? It is safe to say that we do not have any set values for an optimal response in poultry. The nature of an optimal immune response is dependent upon specific conditions (environment, nutritional status, age of bird) and the infection status of the bird. Clearly, a maximum response is not necessarily the optimal response. Because we are aware that the immune response has a cost [14], a moderately effective immune response may provide the greatest responsiveness to an infection. Thus, an optimal immune response to an infection might not be fully immunocompetent but would be immunosufficient or immunoresponsive. Thus, there are 2 keys to evaluating an optimal immune response: (1) measure the response to a pathogen and (2) age of the bird.
CAN INNATE IMMUNITY BE EXPLOITED-MODULATED?

Chickens live in an environment full of microbes. Every day they are exposed to untold numbers of potentially pathogenic agents through the air, water and food they consume, and contact with other birds. Yet despite this exposure, rarely do the birds succumb to infection. The foremost reason for this is the highly efficient early defense response of the innate immune system. The innate host defenses are dedicated to the containment of the pathogens holding infections to a level that can be resolved by the ensuing development of acquired immune mechanisms (Table 1). Innate immunity is the first line of host defenses that entails a series of actions that transpire almost immediately after recognition of an invading pathogen [15]. These actions entail a collection of phylogenetically conserved mechanisms that recognize and respond to the threat of foreign microbes. Upon recognizing pathogen infections, host cellular receptors such as Toll-like and nucleotide oligomerization domain family receptors can trigger a series of signal transduction and gene expression networks (gene programs) to initiate innate immune responses. These innate immune responses can directly control the replication or spread of bacteria and viruses through induction of phagocytosis or antimicrobial products. In addition, the innate immune response can instruct the activation of adaptive immune response through induction of antigen presentation and co-stimulatory molecules. Defects in any steps in the process of innate and adaptive immune responses can increase susceptibility of hosts to pathogen infections, whereas overreactive immune responses can also lead to many inflammatory diseases and metabolic syndromes.

There are definitive functional differences between the innate and acquired immune systems. First, and in terms of comparative immunology, innate is often the only form of host defenses in lower multicellular organisms, including plants and insects. In fact, roughly 98% of all multicellular organisms on earth possess only an innate immune system [15–17] for protection against infections. Acquired immunity is an evolutionary addition to vertebrates [17]. In fact, there are several other characteristics that differentiate innate and acquired immune response including mechanisms of microbial recognition, antigen specificity, speed of activation, generation of a memory response, and self versus nonself discrimination (Table 1).

There are 3 characteristics of innate immune responses (rapid action, limit duration: does not increase with repeated exposure; limited specificity: does not discriminate between pathogens; and ability to be augmented) that we have begun exploiting for targeting the design of immuno-modulatory or antimicrobial compounds for protection or treatment of infections. Because the innate immune response is not pathogen-specific, the ability to stimulate the response in birds is a promising approach of increasing resistance to a variety of pathogens. The one characteristic of the innate response that we have exploited is its ability to be modulated during the first week posthatch. We have shown that the stimulated response results in an increased resistance to Salmonella infections with concomitant increases in heterophil functional activity. We have shown that Toll-like receptor (TLR) agonists all increased the functional immune responses of chicken heterophils (Figures 1 and 2). In addition, using cytosine-guanosine oligodeoxynucleotides (CpG-ODN), the structural components frequently found in bacterial or viral DNA, and the TLR 7 agonist, loxoribine, prevented extraintestinal organ invasion by Salmonella Enteritidis (SE) in neonatal chickens (Figures 3 and 4) [18, 19]. Furthermore, we found that this stimulation was self-limiting, lasting 3 to 5 d after administration of the agonist. These results indicate that the innate immune responses can be augmented and that natural products can potentially be used as antimicrobial compounds.

<table>
<thead>
<tr>
<th>Table 1. Differences in host immune defenses</th>
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<tbody>
<tr>
<td>Innate (constitutive)</td>
</tr>
<tr>
<td>Receptors: patterns</td>
</tr>
<tr>
<td>Nonspecific, defense</td>
</tr>
<tr>
<td>Rapid: immediate, upgrade</td>
</tr>
<tr>
<td>All multicellular</td>
</tr>
<tr>
<td>Self/nonself: indiscriminate</td>
</tr>
<tr>
<td>No memory</td>
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</tbody>
</table>
DO WE WANT A SUSTAINED MODULATION OF THE INNATE RESPONSE TO ACHIEVE GREATER LEVELS OF IMMUNITY?

There is a general assumption among nonimmunologists that more is better when discussing an immune response. However, as discussed previously, more is not necessarily better. We must keep in mind that the optimal immune response is only required during a virulent or pathogenic infection. Thus, the cost of maintaining a sustained stimulation of the innate response is 2-fold: (1) the cost of adding some modulator to

Figure 1. Heterophil degranulation induced by various Toll-like receptor (TLR) agonists. Heterophils (8 × 10⁵/mL) were incubated with various concentrations (mg/mL) of the TLR agonists for 1 h at 39°C. Data represent the mean ± SEM of 3 independent assays for each TLR agonist. Functional differences were determined by ANOVA. Significant differences were further separated by Duncan’s multiple range test. Different lowercase letters above the bars indicate the significant difference (P ≤ 0.05) in β-g-glucuronidase release between the TLR agonists’ stimulated and unstimulated heterophils. LPS = lipopolysaccharide; FLG = flagellin; PGN = peptidoglycan; PAM = palmitoyl-3-cysteine-serine-lysine-4; Poly IC = the synthetic double RNA analog, poly(I:C); LOX = the guanosine analog, loxoribine.
a population of birds would be prohibitive and (2) the costs of maintaining an activated immune system, such as the trade-off in energy needed to support a primed or activated system versus that needed for other energy-requiring physiological functions [14]. Klasing [5] has described the maintenance costs of a control avian immune response as 9% of the birds’ nutrient needs. However, the maintenance costs of an animal with a constant stimulation would conceivably be

Figure 2. Oxidative burst induced by various Toll-like receptor (TLR) agonists. Reactions contained $8 \times 10^5$ heterophils/well, 10 mg of dichlorfluorescein diacetate/mL, and varying concentrations (mg/mL) of the TLR agonists. Plates were incubated for 1 h at 28°C and the relative fluorescent units were measured. Data represent the mean ± SEM of 3 independent assays for each TLR agonist. Functional differences were determined by ANOVA. Significant differences were further separated by Duncan’s multiple range test. Different lowercase letters above the bars indicate the significant difference ($P \leq 0.05$) of relative fluorescent units between the TLR agonists’ stimulated and unstimulated heterophils. LPS = lipopolysaccharide; FLG = flagellin; PGN = peptidoglycan; PAM = palmitoyl-3-cysteine-serine-lysine-4; Poly IC = the synthetic double RNA analog, poly(I:C); LOX = the guanosine analog, loxoribine.
much greater. The expenditures of energy and resources into the immune system surveillance, maintenance of activated cell populations, and production of constitutively expressed protective molecules such as antimicrobial peptides and acute phase proteins in the absence of infection would make resources less available to performance functions. Likewise, costs would arise from the deployment of a primed or stimulated immune system. In addition, it has been shown that a vigorous immune response typically reduces both growth and egg production [13, 14]. Therefore, the additional costs to bird performance would offset any advantages provided by a sustained stimulated immune response.

Another drawback that must be considered when discussing a prolonged stimulation of the innate immune system is that if highly stimulated, the response to any infection would result in excessive inflammation, leading to localized tissue damage and potentially conditions similar to sepsis in mammals. This certainly would be a case of the treatment being worse than the potential infection.

Last, there is one thought that might be considered in this discussion of sustained modulation of the innate immune response. Repeated signaling through pathogen recognition receptors has been reported to result in a reduction in the subsequent proinflammatory cytokine response, a phenomenon known as tolerance. Tolerance is the development of refractoriness to repeated stimulation with the same danger signals and prevents the vicious overstimulation of the innate immune system [20]. More recently, after the identification of the adapter proteins MyD88 and Trif as gatekeepers of 2 distinct signaling pathways initiated after recognition of pathogen-associated molecular patterns, tolerance was shown to affect transcriptional targets of both pathways [21, 22]. Thus, it is possible that a sustained stimulation of the innate

Figure 3. Effect of a synthetic oligodeoxynucleotide containing an unmethylated cytosine-guanosine oligodeoxynucleotide (CpG-ODN) on *Salmonella* Enteritidis (SE) organ invasion. Each experiment consisted of 7 groups with 20 chickens per group: control (SE challenging group receiving no cytosine-guanosine treatment), 25 μg of CpG-ODN #1/chicken, 50 μg of CpG-ODN #1/chicken, 25 μg of CpG-ODN #17/chicken, 50 μg of CpG-ODN #17/chicken, and 50 μg of non-CpG-ODN/chicken. Treatments (0.5 mL) of oligodeoxynucleotides or PBS (for control group) were given by i.p. injection to newly hatched chickens. At 24 h after oligodeoxynucleotide treatments, 0.5 mL of SE (5 × 10⁷ cfu/chicken) was orally gavaged to each chicken. Twenty-four hours after the SE challenge, chickens were killed with CO₂. Liver and spleen were aseptically removed from each chicken and cultured as a combined sample in an enrichment tetrathionate broth overnight (18 to 24 h) at 41°C. After incubation, the broth was streaked on brilliant green agar plates containing carbonicillin-novobiocin (CN) and incubated for an additional 24 h at 41°C. The plates were examined for the presence of CN-resistant SE colonies. The SE colonies were confirmed by appropriate antiserum test [59]. Four independent experiments were conducted at different dates and 80 chickens were used for each treatment group.
immune system could induce a tolerance that would prevent the innate system from reacting against invading microorganisms. More studies will be required to eliminate this as a possible scenario for nutritional modulation of the innate response.

WHEN WOULD MODULATION OF THE INNATE RESPONSE BE APPROPRIATE?

The host immune response to pathogens in the earliest stages of infection is a critical determinant of disease resistance and susceptibility. Unfortunately, neonatal poultry exhibit a transient susceptibility to infectious diseases during the first week of life. This susceptibility is largely due to a qualitative impairment of the avian innate host defenses characterized by a functional inefficiency of heterophils and macrophages for the first 7 to 14 d of life in chickens [20, 23–25]. Because of this deficiency in the functional ontogeny of the avian innate and acquired immune defenses, there is a probable application for potentiating avian host defenses during the first week of life.

In a series of studies, we have shown the ability to stimulate the avian innate immune response during the first week posthatch. In previous studies, the administration of SE-immune cytokines induced protection against salmonellae within 24 h in neonatal poultry [26–29]. Mechanistically, protection was associated with a potentiation of the biological functions of the circulating heterophils including adherence, chemotaxis, phagocytosis, and bacterial killing [30–32]. The cytokine-mediated functional activation of the heterophils lasts about 5 d and coincides with the maturation of natural host
resistance to salmonellosis [32]. Likewise, we have shown heterophil-specific activation with corresponding protection against extraintestinal Salmonella infections in young chickens after either injection of the TLR agonist cytosine-guanosine [18] or adding either highly purified β-glucan [7] or antimicrobial peptides (BT) [33] as feed additives to chickens during the first 4 d posthatch can significantly protect the birds against an SE infection. Chickens fed either the β-glucan or BT-supplemented diet had significantly enhanced heterophil efficacy to phagocytize and kill invading SE (Figures 5 and 6; Tables 2, 3, 4, and 5).

Another period when modulating the innate immune response in chickens would be advantageous is during molting. In the commercial egg-laying industry there is a need for birds to undergo an induced molt to increase the profitability and longevity of flocks. The active laying cycles of flocks can be extended from about 80 to 110 wk or even 140 wk through the use of induced molting [34]. There have been many different methods of inducing molt in commercial laying hens, which include feeding different concentrations of minerals [35, 36], or high-fiber, low-energy diets [37, 38], although the industry-wide standard is feed deprivation for 12 d. However, food deprivation has been shown to have negative but necessary physiological effects on the bird. One of the side effects of molting is the increase of enteric foodborne pathogens into the reproductive tract, leading to contaminated eggs and progeny of infected hens [39, 40]. It has been documented that both the innate and acquired arms of the immune system are negatively affected by feed deprivation [41, 42]. Kogut and colleagues [43] have shown how this negative effect on the innate system dramatically decreased the functional immune response of leucocytes in feed-deprived hens compared with birds fed ad libitum. Currently, there is

Figure 5. Effect of dietary β-glucan on Salmonella Enteritidis (SE) organ invasion. Newly hatched chickens were fed control or β-glucan-supplemented diets for 3 d before SE challenge. At d 4 posthatch, 0.5 mL of SE (10^8 cfu/chicken) was orally gavaged to each chicken. Twenty-four hours after the SE challenge, chickens were killed with CO₂. Liver and spleen were aseptically removed from each chicken and cultured as a combined sample in an enrichment tetraphionate broth overnight (18 to 24 h) at 41°C. After incubation, the broth was streaked on brilliant green agar plates containing carbonicillin-novobiocin (CN) and incubated for an additional 24 h at 41°C. The plates were examined for the presence of CN-resistant SE colonies. The SE colonies were confirmed by appropriate antiserum test [59]. Four independent experiments were conducted on different dates and 80 chickens were used for each treatment group.
increasing interest in using natural products as dietary components to induce molting. Many of the diets being evaluated utilize general nutrient limitations, alteration of the diets’ mineral balance, dietary fillers, and hormones [34, 44]. The specific stressful management practice of inducing a molt by feed deprivation to stimulate multiple egg-laying cycles in hens can be reduced through the use of alternative molting diets [45, 46]. One product that has received atten-

**Figure 6.** Effect of feeding BT cationic peptides on *Salmonella* Enteritidis (SE) organ invasion. One-day-old White Leghorn roosters were randomly distributed into 4 experimental groups, each containing 25 chickens. A control group was fed a control balanced unmedicated corn and soybean meal-based diet. The remaining birds were fed the balanced corn and soybean-based diet that contained either 12 ppm of BT, 24 ppm of BT, or 48 ppm of BT throughout the experiment. Three days posthatch, all chickens were orally challenged with $5 \times 10^7$ cfu/mL of SE + carbonicillin-novobiocin (CN) and maintained on feed throughout the experiment. Four days posthatch (24 h post-SE challenge), all experimental animals were killed. From each chicken, the liver and spleen were aseptically removed, minced, combined in 50 mL of tetrathionate broth for enrichment, and incubated overnight at 41°C. After incubation, the broth was streaked on brilliant green agar plates containing CN and incubated for an additional 24 h at 41°C. The plates were examined for the presence of CN-resistant SE colonies. The SE colonies were confirmed by appropriate antiserum test [59]. Four independent experiments were conducted on different dates and 80 chickens were used for each treatment group. Different lowercase letters represent significant differences ($P < 0.01$) between the heterophils fed the cationic peptide ration when compared with heterophils from chickens fed the control ration. TAMU = Texas A&M University.

**Table 2.** Effect of feeding β-glucan on chicken heterophil phagocytosis

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Heterophils containing SE (%)</th>
<th>Mean number of SE/heterophil</th>
<th>PI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control feed</td>
<td>38.54 ± 0.05</td>
<td>4.38 ± 1.08</td>
<td>172.54 ± 44.92</td>
</tr>
<tr>
<td>β-Glucan feed</td>
<td>78.64 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.20 ± 0.76&lt;sup&gt;a&lt;/sup&gt;</td>
<td>644.10 ± 57.07&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>Means within a column with a superscript letter indicate statistically significant differences ($P < 0.05$) between the heterophils from chickens fed the β-glucan ration when compared with heterophils from chickens fed the control ration.

<sup>1</sup>Heterophils isolated from chickens fed a control diet were compared with heterophils isolated from chickens fed the β-glucan-supplemented diet. Heterophils ($5 \times 10^6$ cells/mL) were incubated with *Salmonella* Enteritidis (SE) at a ratio of 10:1 (SE:heterophil) at 39°C in a 5% CO₂ incubator for 1 h. After this incubation, samples were incubated in ice-cold gentamicin solution (100 μg/mL) in RPMI 1640 medium for 1 h on a rocker platform at 37°C. After the gentamicin incubation, all samples were washed 3 times with RPMI 1640 medium without serum. Five replicate cytopsin smears were made from each experimental sample and examined microscopically. A total of 100 heterophils were counted for each slide. Only bacteria contained within a defined vacuole were counted as being phagocytized by heterophils. Three replicate experiments were performed on different days. The results are expressed as percentage of heterophils with bacteria, mean number of bacteria per heterophil, and the phagocytic index (PI), where PI = (percentage of heterophils containing bacteria) × (average number of bacteria per ingesting heterophil) × 100.
tion because of its relative abundance and low cost is alfalfa. It has recently been shown that combining alfalfa with layer ration induces an effective molt and retains postmolt performance comparable with feed withdrawal [47]. Alfalfa, with its high fiber content, has been shown to have a very long transit time in the gastrointestinal tract of chickens. This increase in transit time favors bacterial degradation of dietary fiber into fermentable substrates such as fructooligosaccharides to short-chain fatty acids. Increasing the fiber content of a diet benefits the digestive system by normalizing colonic function and by increasing fecal weights and evacuation frequency. These actions help maintain the small and large intestine by increasing mucosal structure and function as well as increasing the populations of commensal bacteria in the gastrointestinal tract [48]. We have shown that non-fed birds have a significant reduction in their ability to mount an immune response. Feeding the alternative alfalfa diet did alleviate some of the reductions in immune response observed in non-fed birds (Table 6).

| Table 3. Effect of dietary β-glucan on chicken heterophil degranulation and oxidative burst† |
|-----------------|-----------------|-----------------|-----------------|
| Treatment       | Degranulation (β-glucuronidase released, μM) | Leukocyte oxidative burst (relative fluorescent units × 10⁵) |
| Control feed    | 34.25 ± 1.48a   | 0.06 ± 0.03a    |
| Control feed + OpSE | 124.63 ± 9.38b | 5.09 ± 0.26b    |
| β-Glucan feed   | 37.03 ± 1.38a   | 0.89 ± 0.07a    |
| β-Glucan feed + OpSE | 134.42 ± 12.21b | 10.03 ± 1.05b   |

* *Means within a column with different superscript letters indicate statistically significant differences (P < 0.05) in β-glucuronidase release between the opsonized Salmonella Enteritidis (OpSE)-stimulated heterophils fed the β-glucan-supplemented ration when compared with heterophils from chickens fed the control ration.† Heterophils isolated from chickens fed a control diet were compared with heterophils isolated from chickens fed the β-glucan-supplemented diet.‡ Degranulation by heterophils stimulated by OpSE. Heterophils (8 × 10⁶/mL) were incubated with OpSE (1 × 10⁸ cfu/mL) for 1 h at 39°C. Data represent the mean ± SEM of 3 independent assays. Functional differences were determined by ANOVA. Significant differences were further separated by Duncan’s multiple range test. § Oxidative burst by heterophils stimulated with OpSE. Heterophils (8 × 10⁶/mL) were incubated with OpSE (1 × 10⁸ cfu/mL) for 30 min at 39°C. Data represent the mean ± SEM of 3 independent assays. Functional differences were determined by ANOVA. Significant differences were further separated by Duncan’s multiple range test.

Table 4. Effect of feeding BT cationic peptides on chicken heterophil phagocytosis†

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Heterophils containing SE (%)</th>
<th>Mean number of SE/heterophil</th>
<th>PI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control feed</td>
<td>71.3 ± 1.7⁰</td>
<td>6.01 ± 1.11²</td>
<td>428.51⁰</td>
</tr>
<tr>
<td>Peptide feed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12 ppm of BT</td>
<td>86.6 ± 2.3⁵</td>
<td>9.24 ± 1.41⁵</td>
<td>800.61⁵</td>
</tr>
<tr>
<td>24 ppm of BT</td>
<td>90.4 ± 3.1⁵bc</td>
<td>11.00 ± 1.24⁵</td>
<td>994.40⁵</td>
</tr>
<tr>
<td>48 ppm of BT</td>
<td>93.5 ± 2.6⁵c</td>
<td>11.45 ± 1.32⁵</td>
<td>1,070.58⁵d</td>
</tr>
</tbody>
</table>

* *Means within a column with different superscript letters indicate statistically significant differences (P < 0.05) between the heterophils from chickens fed the cationic peptide ration when compared with heterophils from chickens fed the control ration.† Heterophils isolated from chickens fed a control diet were compared with heterophils isolated from chickens fed the BT-supplemented diet. Heterophils (5 × 10⁶ cells/mL) were incubated with Salmonella Enteritidis (SE) at a ratio of 10:1 (SE:heterophil) at 39°C in a 5% CO₂ incubator for 1 h. After this incubation, samples were incubated in an ice bath for 15 min to stop phagocytosis. The samples were then pelleted and resuspended in ice-cold gentamicin solution (100 μg/mL) in RPMI 1640 medium for 1 h on a rocker platform at 37°C. After the gentamicin incubation, all samples were washed 3 times with RPMI 1640 medium without serum. Five replicate cytospin smears were made from each experimental sample and examined microscopically. A total of 100 heterophils were counted for each slide. Only bacteria contained within a defined vacuole were counted as being phagocytized by heterophils. Three replicate experiments were performed on different days. The results are expressed as percentage of heterophils with bacteria, mean number of bacteria per heterophil, and the phagocytic index (PI), where PI = (the percentage of heterophils containing bacteria) × (average number of bacteria per ingesting heterophil) × 100.
modulation of innate immunity. With the current knowledge of bioactive dietary constituents and modulation of avian immune function [3–5], it is conceivable that commercial diets can be devised immediately that will specifically modulate the birds’ immunity during these windows of high susceptibility to infectious diseases due to less than optimal immune function.

Do Dietary Intervention Strategies Have a Place in Poultry Health?

It has recently been proposed that nutrients and diets can be used as a means to specifically prevent infectious diseases in poultry [5]. In fact, because of the genetically homogenous populations of domestic fowl in the commercial environment, using nutritional dietary intervention strategies could prove to be a cost-effective means to prevent specific infectious diseases and maintain health of flocks of poultry. Klasing [5] suggested that it is feasible to have several feeds available, each with its own immune-modulating nutrient to direct the immune response in a specific direction. Similarly, feeds could be generated for different chicken houses or complexes to generate an optimal response for a given situation. In fact, immunonutrition is not just a concept in human medicine in which specific nutrients or combinations of nutrients are being used as adjunct treatments for surgical, trauma, burned, or critically ill patients as well as protection against cancer development and progression [49–53]. Theoretically, such programs would be easier to introduce into the poultry industry because of the genetically homogenous populations of birds and the intensive rearing systems in place in an industry that can optimize the rational selection of dietary bioactive food.

### Table 5. Effect of feeding BT cationic peptides on chicken heterophil degranulation

<table>
<thead>
<tr>
<th>Treatment</th>
<th>β-Glucuronidase released (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control feed</td>
<td>27.6 ± 4.69</td>
</tr>
<tr>
<td>Control feed + OpSE</td>
<td>83.6 ± 1.37*</td>
</tr>
<tr>
<td>12 ppm of BT</td>
<td>28.37 ± 3.42</td>
</tr>
<tr>
<td>12 ppm of BT + OpSE</td>
<td>110.34 ± 1.51b</td>
</tr>
<tr>
<td>24 ppm of BT</td>
<td>27.47 ± 2.57</td>
</tr>
<tr>
<td>24 ppm of BT + OpSE</td>
<td>118.31 ± 3.34c</td>
</tr>
<tr>
<td>48 ppm of BT</td>
<td>27.59 ± 2.37</td>
</tr>
<tr>
<td>48 ppm of BT + OpSE</td>
<td>125.91 ± 1.41d</td>
</tr>
</tbody>
</table>

*a–dMeans within a column with different superscript letters indicate statistically significant differences (\(P < 0.05\)) in β-δ-glucuronidase release between the opsonized *Salmonella* Enteritidis (OpSE)-stimulated heterophils fed the cationic peptide ration when compared with heterophils from chickens fed the control ration.

1Heterophils isolated from chickens fed a control diet were compared with heterophils isolated from chickens fed the BT-supplemented diet. Degranulation by heterophils stimulated by OpSE. Heterophils (8 × 10⁶/mL) were incubated with OpSE (1 × 10⁸ cfu/mL) for 1 h at 39°C. Data presented are the means ± SEM of 3 independent assays. Functional differences were determined by ANOVA. Significant differences were further separated by Duncan’s multiple range test.

### Table 6. Effects of an alternative molting diet on leukocyte bacterial killing mechanisms in Single Comb White Leghorn layer hens during a 12-d induced molt

<table>
<thead>
<tr>
<th>Day of molt</th>
<th>Treatment group¹</th>
<th>Leukocyte oxidative burst² (mean relative fluorescence)</th>
<th>Leukocyte degranulation² (μM of β-δ-glucuronidase)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>Non-fed</td>
<td>1,941.2 ± 154.27</td>
<td>21.51 ± 0.29</td>
</tr>
<tr>
<td></td>
<td>Full-fed</td>
<td>1,941.2 ± 154.27</td>
<td>21.51 ± 0.29</td>
</tr>
<tr>
<td></td>
<td>Alfalfa-fed</td>
<td>1,941.2 ± 154.27</td>
<td>21.51 ± 0.29</td>
</tr>
<tr>
<td>Day 2</td>
<td>Non-fed</td>
<td>2,575.9 ± 69.38b</td>
<td>28.28 ± 0.63b</td>
</tr>
<tr>
<td></td>
<td>Full-fed</td>
<td>5,960.35 ± 128.89a</td>
<td>43.26 ± 5.98a</td>
</tr>
<tr>
<td></td>
<td>Alfalfa-fed</td>
<td>5,016.1 ± 695.45a</td>
<td>40.02 ± 1.77a</td>
</tr>
<tr>
<td>Day 6</td>
<td>Non-fed</td>
<td>5,946.90 ± 397.41</td>
<td>27.15 ± 1.70b</td>
</tr>
<tr>
<td></td>
<td>Full-fed</td>
<td>7,330.30 ± 732.85</td>
<td>43.74 ± 4.76a</td>
</tr>
<tr>
<td></td>
<td>Alfalfa-fed</td>
<td>6,833.40 ± 972.98</td>
<td>38.93 ± 3.12a</td>
</tr>
<tr>
<td>Day 12</td>
<td>Non-fed</td>
<td>4,324.37 ± 320.39b</td>
<td>36.46 ± 2.84a</td>
</tr>
<tr>
<td></td>
<td>Full-fed</td>
<td>8,537.90 ± 381.13a</td>
<td>48.31 ± 3.54a</td>
</tr>
<tr>
<td></td>
<td>Alfalfa-fed</td>
<td>9,115.10 ± 129.28a</td>
<td>65.53 ± 3.32a</td>
</tr>
</tbody>
</table>

¹Means within a column and day with different superscript letters indicate statistically significant differences (\(P < 0.05\)).

¹Treatment groups represented by non-fed = diet removal; full-fed = complete layer ration; alfalfa-fed = alfalfa crumbles.

²Leukocyte oxidative burst and leukocyte degranulation are represented by the mean of treatment subset (n = 30) with the MS error.
components for flocks, houses, or complexes in specific regions.

Finally, in this era of “-omics,” the publication of the sequence of the human genome, subsequent availability of the avian genome, and the powerful genomic technologies that have been developed over the last 5 to 7 yr, we find that the term nutrigenomics or nutritional genomics has come to the forefront. By its simplest definition, nutrigenomics is the study of nutrient effects on gene and protein expression [54–56]. Accordingly, nutrigenomics research in the context of immunity is an approach to study: (1) the interplay between immune response genes and diet on disease susceptibility, (2) the effects of genetic differences between birds in response to diet (nutrients), (3) the nutritional effect on the expression of immune response genes, and (4) recognition of particular genotypes of chickens in defining an optimal diet. Therefore, nutrigenomics considers nutrition and diet as major environmental factors in the interaction between host response and infectious disease. In recent years, new tools and resources for the analysis of complex traits, such as disease resistance, have been developed in poultry. This started with the creation of genetic linkage maps of the chicken and recently maps based on single nucleotide polymorphisms [57]. These tools have been used to map hundreds of quantitative trait loci for a wide range of traits [58] and summarized in the genetic variation database. The identification of innate immune resistance genes, their mechanisms, and the effect that nutrients or diet have on them also has strategic industrial relevance. Worldwide, the poultry industry faces numerous challenges to remain sustainable. These include the move to more extensive rearing systems and the potential withdrawal of prophylactic and many therapeutic antibiotics. These challenges will all have an effect on both poultry diets and health. It is important that poultry breeders are able to select for genetic improvement in performance when birds are reared in such environments. One obvious phenotype would be immune robustness, and the effect of diet on this robustness is of utmost importance. We need to understand the bird’s immune response to disease to understand which genes might be important in that response and what effect specific immune modulatory nutrients have on this response. Thus, the aim of “-omics” research is to link phenotype, genotype, and QTL whole-genome scans with single nucleotide polymorphism panels and microarrays.

CONCLUSIONS AND APPLICATIONS

1. There is a need to identify practical alternatives to antibiotics, such as immunological interventions and therapeutic antimicrobials, to prevent infectious diseases.

2. Dietary bioactive food components that interact with the immune response have considerable potential to reduce susceptibility to infectious diseases.

3. Nutrition alone cannot be the magic bullet to provide for bird health; this will require a coordinated, integrated approach that combines the research from poultry physiology, metabolism, genetics, and immunology.

4. A maximum immune response is not necessarily the optimal response for the bird. Because the activation of an immune response has a cost, a moderately effective immune response may provide the greatest responsiveness to an infection.

5. The innate immune responses can be augmented and natural products, such as feed additives, can potentially be used as antimicrobial compounds.

6. Definitive windows that provide opportune times for the nutrient modulation of innate immunity would be during the first week of life, especially in broiler chickens because of a decreased immune responsiveness during this time immediately posthatch, and during the molting process in layer-type chickens.

REFERENCES AND NOTES


59. Difco Laboratories, Detroit, MI.