The role of diet in modulating the immune response of broilers: the example of PUFAs

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Summary

Identifying nutritional factors that modulate the immune response and influence an animal’s susceptibility to infectious diseases is currently an active area of investigation. The intercellular and intracellular communication pathways of leukocytes are influenced by dietary meal patterns, plane of nutrition, and high dietary levels of some nutrients. For example, the types and ratios of dietary polyunsaturated fatty acids (PUFA) influence leukocyte function by acting through specific cellular receptors and by changing eicosanoid production patterns. A high ratio of dietary n–3:n–6 PUFA shifts the immune response towards an increased antibody response and at the same time towards a decreased inflammatory response. Decreased inflammatory responses may cause better resilience of broilers during a challenge to some types of diseases but may increase susceptibility to others. Recent studies show that under field conditions, the modulatory actions of dietary PUFAs on the immune system may result in a change in the spectrum of disease problems, with decreased incidence of cellulitis and septicemia, but increased incidence of tumors. Thus dietary PUFAs may be thought of as non–pharmacological immunomodulators that change the incidence of diseases in broiler populations. The decision to include n–3 PUFAs in the diet is dependent upon the specific infectious disease processes that are a problem in a flock at any given time.

Introduction

Minimizing stresses and pathology associated with infectious challenges are primary considerations in optimizing the welfare of animals kept for food production. Optimizing animal health also increases the wholesomeness of the human food supply and improves profits of animal producers. Genetic background (e.g. MHC haplotype), efficacy of vaccination programs and elimination of pathogens by good hygiene are the most important factors in minimizing infectious diseases in chickens. Use of scientifically formulated diets that precisely deliver each nutrient at levels that match the requirement has largely eliminated the overt nutritional deficiencies that impaired immunity and caused greater susceptibility to infectious diseases in the earlier parts of this century. Generally, the dietary level of a nutrient that maximizes productivity of poultry provides sufficient levels for the immune system to function appropriately. In other words, the immune system has a high priority for most of the nutrients needed as substrates for clonal expansion of cell populations and the production of effector molecules such as immunoglobulins (Ig), nitric oxide, complement or cytokines (Klasing 1998). Not surprisingly, nutrition has been seen to be secondary to genetic, vaccination and hygiene approaches to optimizing animal health. Yet, practical experience and epidemiological data demonstrate that an animal’s diet influences its susceptibility to infectious diseases. There is evidence that dietary factors modulate the type, strength, and duration of an immune response by mechanisms that are unrelated to classical substrate roles of nutrients (Klasing 1988; Sklan et al. 1994; Frische et al. 1999). This is because some nutrients and dietary regimens have subtle but important influences on the direction that the immune system takes when it commits to a response.

Immune responses to a pathogen or a vaccine are usually polarized: most of the response is invested in one specific arm and other component systems are relatively non–responsive. For example, a protective response against an intracellular mycoplasmal pathogen is usually a strong cell–mediated response utilizing T–cytotoxic lymphocytes, with minimal response by B–lymphocytes and low levels of immunoglobulin (Ig) production. However, genetic, environmental and nutritional factors can bias immune responses towards B–cell–mediated Ig responses with little engagement by T–cytotoxic cells. In cases where a T–cytotoxic response is protective, nutritional factors that modulate the immune response towards an antibody response cause greater morbidity. Other disease organisms, E. coli for example, are best combated by Ig responses, and nutritional factors that polarize the immune response...
away from T–cytotoxic responses and towards Ig responses are beneficial. Obviously, the tilting of immune responses by nutritional modulators results in better protection against some types of pathogens at the expense of greater vulnerability to other pathogens. The challenge facing nutritionists is to determine the details of the changes in the balance of the immune response induced by immunoregulatory nutrients so that they can be utilized at times when they will be helpful in defence against current disease problems, and not used when they might be contra–indicated.

The key point is that the regulation of the complex network of immune cells is changed by diet so that the default response of the immune system is slightly modified. Whether this modulation is of benefit or not is context dependent. Dietary manipulations that increase antibody responses and decrease inflammatory responses would be classified as beneficial in the context of facilitating good vaccination responses and resistance to some types of infections—but they could be classified as contraindicated for novel disease challenges where the best defense is a robust inflammatory response. Obviously, nutritionists must be vigilant and critically question claims that a nutrient ‘improves immunity. No known nutrient bolsters all aspects of the immune response and it is doubtful that this sort of bioactivity would be useful for animal production even if it were possible as productivity decreases with the frequency and vigor of immune responses (Roura et al. 1992; Spurlock 1997).

A variety of studies in chickens and laboratory rodents indicate an immunomodulatory role of several nutrients at levels that are considerably greater than current requirement recommendations (NRC), which are based on the prevention of deficiency related pathology. This report will describe the mechanistic bases and the practical applications of immunomodulation via diet using specific examples from research on dietary PUFA.

### Methods

Six types of experiments are needed to ascertain the balance between the positive and negative attributes of an immunomodulatory nutrient or feeding regimen (Table 1).

#### Type 1

Laboratory experiments with in vitro cultures of leukocytes are used to define the mechanisms by which the nutrient affects functional properties of immune cells. The existence of nutrient receptors or second messenger pathways that are involved in the regulatory actions are detailed and an analysis of the effected genes and gene products is made. Much of this research has been conducted by the basic medical sciences community utilizing mammalian and, on occasion, avian cell lines. Obviously the immediate applicability of these data to the producer is negligible. However, basic mechanistic research using transformed cell lines underlies the theoretical basis for most of the commercially viable applications of nutritional immunomodulation. Without a sound appreciation for the basic mechanisms that a nutrient affects the responses of specific leukocyte populations, it is difficult to use nutritional modulation as a management tool for ameliorating specific disease problems.

#### Type 2

Feeding studies conducted in very defined conditions (e.g. batteries, controlled environment) are made to determine the dose response relationship between the nutrient and indices of immune function. Typically a panel of in vivo assays are run that examine the components of specific and innate immunity. It is important that these experiments are done in commercially relevant chickens and are repeated in time

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Table 1  Types of experiments needed to ascertain the effect of an immunomodulatory nutrient or feeding regimen on immunocompetence and disease resistance.

<table>
<thead>
<tr>
<th>Type of Experiment</th>
<th>Number of chickens</th>
<th>Experimental control</th>
</tr>
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<tbody>
<tr>
<td>1. Identification of mechanisms</td>
<td>cells from 1 animal</td>
<td>++ + + + +</td>
</tr>
<tr>
<td>2. Feeding studies characterizing affected</td>
<td>50 – 500</td>
<td>++</td>
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<tr>
<td>cells and processes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Feeding studies identifying impacts on</td>
<td>50 – 500</td>
<td>+++</td>
</tr>
<tr>
<td>disease resistance using defined challenges</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Feeding studies using natural challenges</td>
<td>1000 – 5,000</td>
<td>+++</td>
</tr>
<tr>
<td>5. Field-tests in actual production facilities</td>
<td>50,000 – 1,000,000</td>
<td>++</td>
</tr>
<tr>
<td>6. Correlation analysis using large data–bases</td>
<td>&gt;10^8</td>
<td>–</td>
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</table>

^1^The level of control of experimental variables that the investigator has over the experimental protocol ranges from very high (++++) to very little (–).
because a plethora of environmental and laboratory phenomena can effect the results in any given experiment. The literature is replete with instances where nutritional influences on various immunoassay endpoints can not be replicated between laboratories or even within a laboratory at different times. Careful attention to experimental design, minimizing the stress of taking measurements and awareness of the physiological relevance of the assays are extremely important. Often, acute experimental artifacts, such as the accumulated stress resulting from the order that birds are captured, injected, bled, etc. can have a confounding effect on the results unless experimental design is stringently controlled. It is also useful to use in vivo assays to the extent possible. This is because in vitro assays utilize cell isolation procedures, artificial culture media and heterologous sera that separate the treatments (nutrients delivered by diet to a living bird) from the cells involved in the assay, seriously compromising interpretation of the results.

Controlled feeding studies must also consider the costs in terms of productivity of modulating the immune system. The negative impact of an authentic disease challenge or an inappropriate immune response to an unimportant challenge is well documented (Klasing and Korver 1997; Klasing et al. 1988). It is obvious that any effort to improve immunocompetence should not compromise the productivity of healthy birds. Birds that are not challenged by pathogens still engage in immune responses against commensal microflora on their epithelia. Dietary treatments that increase responses to all challenges, including those to non–pathogenic commensal microflora, may have the unintended effect of decreasing productivity in pathogen–free flocks.

Type 3

Experiments examining the impact of immunomodulatory levels of the nutrient on the susceptibility, morbidity, and mortality of poultry following challenges with specific pathogens are required. Model disease systems should utilize challenging organisms that span the spectrum of diseases protected by innate defense mechanisms (i.e. inflammatory responses), Th1 responses (cell mediated immunity), and Th2 responses (some types of Ig–mediated immunity). Well–characterized and repeatable challenge models that span this spectrum include coccidiosis, salmonellosis, and colibacillosis, respectively.

Type 4

Trials conducted on experimental farms should examine the impact of the nutrient or feeding regimen on morbidity and mortality of naturally occurring disease problems. The exact number of chickens per treatment required for such a test is dependent upon the level of infectious challenges. Often experimental grow–out houses are over–managed or too small for the purposes of this test. Sometimes the facilities that are owned and operated by producers permit more authentic conditions and disease challenges than those operated by Universities, which must adhere to strict and artificial conditions of animal care and use. Still, more than a 1,000 birds per treatment group are usually needed in order to detect statistically meaningful differences in morbidity and mortality due to dietary immunomodulation.

Type 5

Field–tests in actual production facilities that provide real–world challenges are required. These tests superimpose a variety of production stresses on multiple and often undefined challenges from infectious diseases. These tests require diligent record keeping at various grow–out locations and slaughter facilities. Oversight of the operations at all levels in the experimental chain is critical (i.e. feed mill, grow out facility, slaughter house). At this level of investigation the variability introduced by economic, environmental, and human factors is enormous and can only be corrected for by very large replications of animals, locations, managers, and time. The condemnation records taken by the government inspectors at the processing plant are an invaluable source of information on morbidity due to defined disease processes.

Type 6

Lastly, the collection of a plethora of data that may serve a common purpose from as many sources as possible and examining these data with very conservative statistical algorithms permits correlation analysis on a massive scale. For example, in the United States several companies collect a wide variety of information from the major poultry integrators, including records on feed nutrient levels, live performance, mortality, and condemnation rates according to cause. Correlation analysis of feed nutrient levels with mortality, morbidity, and condemnation rates indicates optimal levels of specific nutrients that maximize productivity and minimize mortality and morbidity.

Results and discussion

The immunomodulatory actions of different families of PUFAs have been examined in a range of studies. These investigations range from the molecular analysis of PUFAs receptors and their effects on gene activation in leukocytes to disease resistance studies using field tests in actual production facilities.

Mechanistic studies

Leukocytes have receptors for and are regulated by retinoic acid, 1,25–dihydroxyvitamin D3, and PUFAs. The receptors for PUFAs include PPAR–α located in the cytoplasm and PPAR–γ located in the nucleus. Ligand binding by PPAR–α in macrophages decreases their
pro-inflammatory propensity by inhibiting the transcriptional activity of the NFkB p65/RelA subunit, decreasing responsiveness to tumor necrosis factor–α (tumor necrosis factor) and interferon–α (Chinetti et al. 1998). Ligand binding to PPAR also inhibits inducible nitric oxide synthetase, further inhibiting the inflammatory response (Colville–Nash et al. 1998). Dietary PUFAs may also influence the response of leukocytes to regulatory cytokines, hormones and antigens through influences on post–receptor signaling. The C18, C20, and C22 PUFAs are preferentially incorporated into diacylglycerol phospholipids of cell membranes. Diets rich in n–3 fatty acids result in increased occupation of the sn–2 position by n–3 PUFAs like eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) at the expense of arachidonic acid. The release of PUFAs from membrane phospholipids is an important part of the second messenger cascade of many communication molecules such as hormones and cytokines that regulate immune responses. Following release catalyzed by the enzyme phospholipase A2, PUFAs are further metabolized by one of several enzymatic pathways to form eicosanoids. The cyclooxygenase pathway results in the production of the prostaglandins (PG) and the thromboxanes, whereas the lipoxigenase pathway results in the production of the leukotrienes (LT).

A relationship between the type of dietary fat and the rate of prostaglandin production has been demonstrated in chickens (Fritsche and Cassity 1992; Watkins 1995). The exact form of the end product eicosanoid and its potency is determined by the species of PUFA that served as the precursor. Prostaglandins of the E series (PGE) are important in mediating the effects of pro–inflammatory cytokines, such as the induction of fever, skeletal muscle catabolism, and anorexia. Leukotrienes of the B series (LTB) are potent chemoattractants, which draw phagocytic cells to sites of inflammation. Arachidonic acid (n–6) is the precursor fatty acid of PGE2, and LTB4, while EPA (n–3) acid is the precursor of PGEl2 and LTB5. In many situations, the latter two eicosanoids are much less potent in activity than eicosanoids derived from arachidonic acid and thereby increase the intensity of the inflammatory response.

Both the amounts and types of eicosanoids released have important consequences for the immune and inflammatory responses. Many of these effects are mediated by the amounts of pro–inflammatory cytokines such as interleukin–1 (IL–1), IL–2, IL–6, TNF and interferon–γ that are released during a response. For example, as the dietary n–3:n–6 ratio increases, PG synthesis decreases and the inducible production of IL–1, IL–2, IL–6 and TNF is suppressed (Meydani and Dinarello 1993). Eicosanoid production mediates many of the actions of cytokines and dietary fat can modulate the actions of cytokines once they are released. As basic nutrition research continues to characterize the mechanisms through which PUFAs modulate cellular communication, developments in poultry nutrition put these developments in practical perspective.

**Feeding trials**

A number of investigations have demonstrated modulation of the immune response by the type of fat incorporated into the diet. Fritsche and colleagues (Fritsche and Cassity 1992; Fritsche et al. 1991; Fritsche et al. 1991) fed high levels of various oil sources and found that the humoral immune response to sheep red–blood cells was higher in broiler chicks fed fish oil (a rich source of n–3 PUFA) than in chicks fed lard, corn oil, or canola oil, but cell mediated responses were depressed. Interestingly linseed oil, which is high in γ–linolenic acid (C:18, n–3) but not the longer chain n–3 PUFAs, did not have the same effect as fish oil. In fact, linseed oil decreases the antibody response in some genetic strains (Parmentier et al. 1997). Friedman and Sklan (1995) have demonstrated that high dietary levels of linoleic acid (C:18, n–6) impair the antibody responses by increasing the time required for response development, decreasing the maximum titer of antibody, and accelerating the decline in the response. Conjugated linoleic acid (c–9,t–11–octadecadienoic acid) displaces arachidonic acid in phospholipids and also increases antibody responses but decreases the systemic inflammatory response (Miller et al. 1994). Replacement of dietary corn oil with low levels of fish oil (1.5–2%) causes a reduced inflammatory response to bacterial lipopolysaccharides as indicated by fever, anorexia, acute phase protein synthesis, and the release of the pro–inflammatory cytokines IL–1 and tumor necrosis factor. In general, experiments on the influence of dietary fish oil as a source of n–3 PUFAs on the immune response of broiler chickens have produced results similar to those found in humans and laboratory rodents (Korver and Klasing 1997; Korver et al. 1998). The modulatory action of n–3 PUFA on the immune system is mediated by a shift in the types of responses by antigen–stimulated T–helper lymphocytes (Fritsche et al. 1999). Dietary n–3 PUFA result in T–helper cells that release low levels IL–12 and gamma–interferon, indicating a shift in T–cell help to a T–helper type–2 phenotype. This shift in cytokine secretion by T–cells influences B lymphocytes to respond to antigen with greater antibody production but decreases the responses of T–cytotoxic cells and inflammatory macrophages.

**Challenge studies**

The influence of dietary PUFAs on the resistance of chicks to coccidiosis has been investigated. Menhaden oil (a rich C:20 and C:22 n–3 source) at 4–5% of the diet decreases the morbidity associated with a challenge with *Eimeria tenella* (Figure 1) and 5% of either menhaden oil or linseed oil decreases lesion scores associated with mild *E. maxima* infections (Allen et al. 1998; Allen et al. 1996; Korver et al. 1997). Though
immunomodulatory actions of n–3 PUFAs are probably involved in increased protection, other mechanisms may also play a role. The n–3 PUFAs may cause increased oxidation of oocysts or increased fragility of intestinal epithelial cells that causes premature release of parasites into the caecal lumen (Danforth et al. 1997). Certainly we must gain a greater understanding of the immunological defences necessary for protection from the myriad of other infectious diseases experienced by poultry and for the specific ways that nutritional modulation affects these defences.

The systemic component of an inflammatory response is thought to be the primary component of an immune response that impinges on the productivity of poultry (Klasing and Korver 1997). This response is mediated by pro–inflammatory cytokines (e.g. IL–1 and TNF) and by corticosterone. An analysis of the cytokines released during an antibody response suggests that this arm of the immune system causes less impairment in productivity than the inflammatory response. Dietary factors that shift the immune response from inflammatory toward antibodies would theoretically ameliorate losses in productivity associated with infectious diseases. This process is known as resilience. Several experiments indicate that n–3 PUFAs improve the resilience of broiler chickens during a bacterial challenge simulated with bacterial lipopolysaccharide (LPS) or controlled challenges with coccidia (Klasing and Korver 1997). Diets containing 1–2% menhaden oil permit faster growth during a challenge with Salmonella typhimurium LPS or heat–killed Staphylococcus aureus relative to diets with the same amount of corn oil. Expression of IL–1 is decreased in chicks ingesting fish oil–based diets and this is reflected in less fever and lower circulating levels of hemopexin, an acute phase protein and index of an inflammatory response. These results indicate that the ratio of n–3:n–6 fatty acids in the diet may affect growth rates during infectious challenges by decreasing the amount of nutrients redirected from skeletal muscle accretion to host defense mechanisms.

Feeding studies using natural challenges

Field tests in actual production facilities have been conducted in poultry (Klasing and Korver 1997) and provide an opportunity to examine how the immunomodulatory actions of PUFAs actually translate into changes in the incidence of natural infections. For example, a trial with 960,000 chicks was conducted at a single commercial production facility that compared two diets differing only in their content of n–3 and n–6 PUFAs. In this trial, disease incidence was determined from the records of the USDA inspector who observed the health of birds at slaughtering and processing. The diet enriched in n–3 PUFA significantly decreased the incidence of septicemia from all causes by 25% and the incidence of cellulitis, which is usually due to E. coli–induced inflammation of scratches in the skin, by 18%. Conversely, the incidence of tumors, both spontaneous and from Marek’s Disease Virus, increased by 24%. This study suggests that the modulatory actions of dietary PUFAs on the immune system result in a change in the spectrum of disease problems that correlate with their actions identified in mechanistic and smaller–scale feeding studies. Thus dietary PUFAs may be thought of as non–pharmacological immunomodulators that change the incidence of diseases in animal populations. The decision to include a n–3 PUFA source in the diet is dependent upon the prevailing infectious of disease problems in the flock. If the most problematic infectious agent is controlled by

Figure 1  The effect of 4% dietary fish oil or corn oil on broiler weight gain and the inflammatory cell infiltration into the ceca following a challenge with Eimeria tenella (Korver et al. 1997).
antibody responses then dietary n–3 PUFA may be advantageous, whereas n–3 PUFAs might be contra–indicated for disease problems that require a strong cell–mediated immune response for control.

References


