POULTRY IMMUNITY RESPONSES TO DIETS CONTAINING FISH OIL

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The purpose of the immune system is to protect the body from pathogens. When an animal is exposed to pathogens, a series of physiological and behavioral changes occur as part of the acute phase of the inflammatory response. These changes can lead to decreased feed consumption, slowed growth rate, and even the loss of skeletal muscle. Immunogens (anything which elicits an immune response, whether pathogenic or non-pathogenic) can elicit an immune response even in the absence of any danger of infection to the animal. In the case of non-pathogenic immunogens, or low-level pathogenic challenges, the activation of the inflammatory response can result in decreased productivity of the animals. Thus, immune responses are not always beneficial to the health of the animal. An excellent example of this is autoimmune diseases such as rheumatoid arthritis. In this disease, the immune system (phagocytic cells) attack the body's own cartilage. Clearly, an increase in this response is detrimental to the health of the individual. Likewise, inappropriate inflammatory responses to non-pathogenic immunogens may impair the health and the growth of an animal. Therefore, it is important to optimize, rather than maximize the immune response.

Inflammation is an early, non-specific response to injury or infection. This response plays an extremely important role in dealing with an invading immunogen the first time that specific stimulus is encountered. The phagocytic cells of the inflammatory response (such as macrophages) process the immunogen and present it to the cells of the specific immune system.

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The specific (cell-mediated and humoral) immune system then can develop memory to that particular immunogen such that the next time it is encountered, a swift, specific response can be mounted with a greatly reduced requirement for the inflammatory response. Therefore, the inflammatory response is essential for the immune system to "learn" about new immunogens.

During the early stages of an inflammatory response, the liver produces a large amount and number of proteins involved in host protection. These proteins, including metallothionein and hemopexin, act non-specifically to create an environment which is inhospitable to pathogenic organisms. Metal ions are withdrawn from circulation and stored so that they are unavailable to the pathogens. Increased body temperature, alterations in the way nutrients are partitioned within the body and other physiological changes are used to combat the perceived threat. If the perceived threat, however, is a non-pathogenic immunogen which is incapable of causing disease, the acute phase inflammatory response serves no purpose, and will only lead to discomfort and poorer performance of the animal.

Nutrition plays an important role in modulating the immune status of the animal, not only in malnutrition, which can impair the ability of an animal to mount an immune response, but via individual nutrients within a complete and balanced diet which can alter the way the body responds to stimulation of the immune system. During an immunological challenge, the immune system preferentially receives nutrients over other body systems. For example, lipid metabolism is altered to provide energy, and skeletal muscle is catabolized (cachexia) to provide amino acids to the immune system. The way in which nutrients are partitioned to the various functions of the body is also altered. For example, the intestines and organs involved in the immune response such as the liver and spleen increase, and skeletal muscle decreases, as a proportion of body mass.

When an animal is exposed to constant non-pathogenic or low level pathogenic challenges, the immune system and the inflammatory response are constantly diverting nutrients away from growth. Even though the animal is not overtly diseased, its growth rate is lessened because of the activity of the immune system.

The environment in which the animals are raised can affect their physiology. In extremely clean environments, broilers were observed to have growth rates 10 to 15% higher than those raised in conventional environments. This was true even in the absence of overt pathological infections. As noted previously, exposure to non-pathogenic or low level pathogenic immunogens can cause an immune response. The immune system releases signals and mediators which can decrease growth rate and even cause the loss of skeletal muscle protein.

Antibiotics can be effective in improving growth rate because they minimize the interaction between a pathogen and the immune system of a bird. Experiments show that when antibiotics are fed to birds in a dirty environment, growth rate is increased. However, when the same antibiotic is fed to birds in an extremely clean environment, there is no improvement in growth rate.

In most cases the pathogens which are most likely to result in overt disease are controlled by mechanisms which do not require a prolonged inflammatory response. For example, many viral diseases are avoided by vaccination, and coccidial infections are averted by the inclusion of anticoccidial drugs in the feed. Therefore, it may be possible to decrease the responsiveness of broilers to inflammatory immunogens without affecting the health of the bird.

The goal of the current research is to attenuate the inflammatory response such that it is less responsive to non-pathogenic immunogens, yet responsive enough to avoid pathological

situations. As well, the specific responses of the immune system should remain intact or even be enhanced. Stated briefly, the inflammatory response should be a measured, local response, and should involve the specific immune response as quickly as possible.

An inflammatory response can be elicited experimentally by injecting non-pathogenic immunogens such as heat-killed *Staphylococcus aureus* or bacterial lipopolysaccharide (LPS). Experimentally, these can be used to model infectious diseases or non-pathogenic stimulation of the immune system. These immunogens are not infectious, but possess the very factors which the immune system responds to on the corresponding live pathogens. This allows for a very controlled, uniform stimulation of the immune system within experimental groups. As the compounds are not infectious, there is no danger that one animal will develop a greater infection than another, as can be the case when uniform doses of infectious agents are given to different animals.

Research *in vitro* has shown that feeding n-3 polyunsaturated fatty acids (PUFA) as fish oil increases the levels of n-3 PUFA and decreases n-6 PUFA of macrophages (German *et al*, 1988). This is important because of the role of macrophage membrane fatty acids in responsiveness to immune challenges.

Eicosanoids are metabolites of 20-carbon polyunsaturated fatty acids (PUFA) which mediate the immune response via their role in cell-cell communication. Eicosanoids include prostaglandins (PG), leukotrienes (LT) and thromboxanes (TX). The precursor fatty acids of eicosanoids can only be obtained from the diet. As a result, the abundance of these precursors in the tissue is proportional to the abundance in the diet. Eicosanoid precursor fatty acids are incorporated into cell membranes, and are released by the action of phospholipases in response to

various signals, including signals from the immune system. The released fatty acids can then be metabolized by the cyclooxygenase (CO) pathway into prostaglandins, and via the lipoxygenase (LO) pathway into leukotrienes. Prostaglandins of the E series (PGE) are important in mediating the effects of proinflammatory cytokines, such as the induction of fever and anorexia. Leukotrienes of the B series (LTB) are potent chemoattractants which draw phagocytic cells to the site of inflammation. Arachidonic acid is the precursor fatty acid of PGE_2 and LTB_4, while eicosapentaenoic acid (EPA) is the precursor of PGE_3 and LTB_5. The latter two eicosanoids are much less potent in activity than eicosanoids derived from arachidonic acid.

Cytokines are hormone-like peptides which are involved in modulation of the immune response and cell-cell communication. Proinflammatory cytokines include Interleukin-1 (IL-1), which is responsible for fever induction and anorexia; interleukin-6 (IL-6), which is involved in proliferation, differentiation and maturation of various immune cells, as well as being especially important in initiation of the acute phase response. Tumor necrosis factor (TNF) is responsible for the cachexia associated with inflammation.

Research indicates that cytokine activity and production and eicosanoid metabolism are closely related. Dietary n-3 PUFA reduced the pyrogenic (fever-inducing) effect of peripheral and brain injections of IL-1 in the rat. It was suggested that this effect was due to the action or release of PG (Cooper and Rothwell, 1993). As the dietary n-3:n-6 ratio was increased, PG synthesis was decreased (Hardarottir and Kinsella, 1992). Dinarello *et al* (1993) suggest that n-3 PUFA decrease the response to IL-1 via decreased cyclooxygenase metabolites, as well as the production of IL-1 via metabolites of the lipoxygenase pathway. Dietary n-3 PUFA suppressed the inducible production of IL-1, IL-2, IL-6 and TNF, associated with a decrease in PGE_2

(Meydani *et al*, 1991). Drugs which block eicosanoid metabolism also tend to decrease the production of pro-inflammatory cytokines. Decreased PGE_2 production caused by a cyclooxygenase inhibitor or by altering the ratio of cell membrane n-3:n-6 reduces the anorexia induced by IL-1 (Hellerstein *et al*, 1989). Eicosanoid production and activity is mediated by cytokines; activation of target cells by IL-1 causes the production of eicosanoids, especially PGE_2. This eicosanoid can also be used to regulate the production of IL-1 by macrophages (Kunkel et al, 1986).

The ratio of n-3:n-6 PUFA may be more important in altering immune responsiveness than the total level of dietary n-3 PUFA. For example, decreased PG synthesis by the spleen was due to the proportion of n-3 and n-6, rather than the total PUFA (Lokesh *et al*, 1986). One study found no changes in lung, liver or platelet arachidonic acid or its eicosanoid metabolites in response to increasing the amount of n-3 PUFA in the diet when the ratio of n-3:n-6 PUFA was held constant (Boudreau *et al*, 1991). Another group found that at high levels of 18:2n-6, even a large amount of dietary fish oil failed to reduce eicosanoid production (German *et al*, 1988).

Dietary fish oil may be one way to achieve some of the previously mentioned modifications of the inflammatory response. Cold-water marine fishes yield an oil which is high in n-3 PUFA. Numerous studies show that the inflammatory response to immunogens can be decreased by feeding fish oil. In addition, several studies indicate that specific immunity may be enhanced.

The n-3 PUFA in fish oil may be responsible for decreasing the production and potency of eicosanoids in many tissues, including skin, splenocytes, platelets and various phagocytic cells. Plasma levels of inflammatory cytokines and acute phase proteins have been shown to decrease

after immunogen treatment of fish oil-fed animals. Indices of specific immunity in these animals are either unchanged or even improved. Dietary fish oil resulted in birds having higher antibody titers to sheep red blood cells than those fed lard, corn or canola oil-based diets (Fritsche *et al*, 1991). The mechanisms involved in these alterations appear to be those discussed earlier in this presentation, i.e. decreased inflammatory response due to changes in the production and potency of mediators of inflammation.

Dietary fish oil can be effective in improving the growth of broiler chickens. Chicks fed fish oil diets had greater body weight gains than those fed either lard, corn oil or canola oil (Fritsche *et al*, 1991). We have repeatedly seen improvements in broiler performance when fish oil has been included in the diet. Diets containing up to 2% fish oil resulted in 9 to 10% greater body weights than diets containing up to 2% corn oil in non-challenged chicks, and 13% greater body weights in LPS-challenged chicks (Korver and Klasing, 1994). Interleukin-1 expression was decreased in fish oil-based diets, and there was a source by level interaction for hemopexin. Hemopexin, an acute phase protein and index of an inflammatory response was increased when 2%, but not .5% corn oil was fed *vs*.the respective levels of dietary fish oil (Korver *et al*, 1994). When chicks were fed 4% of either fish or corn oil and infected with coccidia, fish oil addition to the diet resulted in a nearly significant ($P<.06$) 8% increase in weight gain and increased feed consumption by 4% *vs* corn oil diets. Coccidial infection had no effect on body weight gains of chicks fed fish oil, however chicks fed corn oil and challenged with coccidia had 6% lower body weight gains than unchallenged corn oil-fed birds (Korver and Klasing, 1995a). In the most recent experiment, chicks were fed either 3, 6 or 9% supplemental oil and an n-3:n-6 PUFA ratio of either .07, .33, .66 or 1 in a 3 X 4 factorial design. Body weight gains over the course of the

experiment were 6% greater for diets containing the highest n-3:n-6 ratio than for the lowest ratio, independent of dietary fat level. At the 9% level of supplemental fat, IL-1-like activity released from splenic leukocyte supernates was 69% lower for the diet having an n-3:n-6 ratio of 1 than the diet having a ratio of .07. The highest level of IL-1-like activity was seen in the 3% fat/.07 ratio diet, and was 75% greater than that of the 9% fat/1 ratio diet which had the lowest activity. These results indicate that IL-1 release is affected by alterations in the ratio of n-3:n-6 PUFA in the diet (Korver and Klasing, 1995b).

In summary, the inclusion of fish oil in the diets of animals may improve growth rates by decreasing the amount of nutrients redirected from skeletal muscle accretion to the immune system. Research is unclear regarding the effects of dietary fish oil under conditions of severe pathogenic challenges, but initial results indicate that the health and performance of animals under normal conditions may be improved by the addition of fish oil to the diet.

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