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Nutritional Immunomodulation as an Approach to Decreasing the Negative Effects of Stress in Poultry Production

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Nutritional Immunomodulation As an Approach to Decreasing the Negative Effects of Stress in Poultry Production

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Abstract

Stress can lead to changes in the immune response resulting in both increased and decreased resistance to opportunistic bacterial pathogens. Growth-promoting antibiotics have been a major tool in modulating host-pathogen interactions and limiting clinical and sub-clinical bacterial infection in confined animal production. Regulatory pressures to limit antibiotic use in poultry production and recent international marketing agreements that prohibit treating poultry with antibiotics have limited the disease-fighting tools available to poultry and livestock producers, particularly in Europe. There is a need to evaluate potential antibiotic alternatives to improve both production and disease resistance in high-intensity food animal production. Nutritional approaches to counteract the debilitating effects of stress and infection may provide producers with useful alternatives to antibiotics. Improving disease resistance in food animals, particularly in the absence of antibiotic treatment, is a key strategy in the effort to increase food safety. ARS research has demonstrated the efficacy of several nutritional immunomodulators, including vitamin D_3 and yeast cell wall products, to protect against bacterial infection due to stress and challenge with opportunistic pathogens. These studies also provide an animal model for testing the efficacy of nutritional strategies that may affect the response to stress and related infection in humans.

Introduction

The relationship between stress and chronic disease has been difficult to establish due to the fact that stress can both increase and decrease disease resistance based on many interacting factors including the type and degree of stress as well as the individual perception of, or response to, the stressor (Biondi and Zannino 1997, Glaser et al. 1999, Salak-Johnson and McGlone 2007). This necessitates the use of animal models in which there can be a high level of control of both environmental and genetic factors to study the effects of stress and methods to modulate those effects.

The stress response has been implicated as the rate-limiting factor leading to ageing and senescence (Johnson et al. 1996) and is an important factor in susceptibility to infection (Glaser et al. 1996). The prospect of modifying the stress response of humans using nutritional supplementation has also been suggested (Romeo et al. 2008). While the human nutraceutical market presents many products that claim to improve the stress response, very little peer-reviewed research has been published in this area. In humans, psychological stress, exercise stress, and sleep deprivation models have been used to demonstrate the effects of stress and to study nutritional immuno-modulators (Hamer et al. 2004). However, the great degree of variability in the stress response requires the use of a large number of individuals and the ability to control and/or manipulate the environment.

Animal models of the stress response have historically utilized rodent species. However, a large body of data is being generated in the animal agricultural sciences due to the need to evaluate potential antibiotic alternatives to improve disease resistance in high intensity food animal production.

Antibiotics are primarily used to compensate for the high levels of stress that can be present in intensive animal production. Stress can decrease growth and feed conversion efficiency and change the immune response. We have been investigating a number of different approaches to modulate the stress response of turkeys, including nutritional approaches.

Growth promoting antibiotics are thought to function mainly by changing the intestinal bacterial flora. Another mechanism by which they improve production values may be by their ability to decrease chronic disease incidence in animals, thereby lowering the level of immunological stress (Roura et al. 1992). The stresses of intensive poultry production can lead to changes in the immune response that lead to decreased resistance to infection with opportunistic pathogens. Our research program, using a respiratory disease challenge model, has allowed us to study nutritional strategies for increasing both disease resistance and production values in turkeys and broiler chickens.

This paper will review the results of three previously published studies utilizing nutritional immunomodulation to counter the effects of stress in poultry production.

The objective of Experiment 1 (Huff et al., 2000a) was to determine the effects of water supplementation with vitamin D3 (High D3, I.D. Russell Co., Longmont, CO) on disease resistance in a turkey osteomyelitis complex (TOC) challenge model using immunosupression with the synthetic glucocorticoid, Dexamethasone (Dex) (Huff et al. 1998, 2000b).

The objective of Experiment 2 (Huff et al., 2002b) was to determine the effects of dietary supplementation with β-glucan on production values of *E. coli* challenged turkey poults.

The objective of Experiment 3 (Huff et al., 2007) was to evaluate the ability of a brewer's yeast extract feed additive (Alphamune™, Alpharma Animal Health Division) to protect against the effects of an *E. coli* respiratory challenge in a cold stress model.

Materials and Methods

Experiment 1. One hundred twenty male poults were assigned to 8 pens in a completely randomized experimental design with two pens of 15 birds/pen for each treatment x challenge group. Half of the birds were provided drinking water treated with 2064 IU of Vitamin D_3 for the first 5 days after hatch followed by 4128 IU/L for 12 hours before and after stressful events, which included weighing and Dex treatment. Challenged birds were treated with Dex at 5 weeks and again at 10 weeks of age. All survivors were necropsied at 13 weeks of age.

Experiment 2. One hundred sixty day-old male turkey poults were wing-banded and placed into brooder battery pens in a completely randomized design with two pens of 8 birds/pen for each treatment. Diets were supplemented with 0, 10, 20, 40, or 80 g/tonne of a highly purified β-glucan feed supplement (Immustim®, Immudyne, Inc., Houston, TX 77042), which was fed continuously.

At 4.5 wks of age half of the birds were challenged with an airsac injection of 50-100 cfu of *E. coli* and were necropsied 2 wks later.

Experiment 3. One hundred eighty birds were weighed by pen, wing-banded, and placed into randomly assigned brooder battery pens. There were 6 treatments with 3 replicate pens of 10 birds/pen for each treatment in a 3 × 2 (3 feed treatments × 2 challenges) experimental arrangement. Poults were fed a standard unmedicated turkey starter diet or the same diet supplemented with either 1 lb/ton (504 g/tonne) or 2 lb/ton (1008 g/tonne) of a brewer's yeast extract feed additive (Alphamune™, Alpharma Animal Health Division), that combines both the immunomodulatory properties of (1,3)/(1/6) β-glucan with the performance enhancement of mannan-oligosaccharide. Challenged birds were exposed to intermittent cold stress (12-16^{o}C) during wk 1-3 (Table 1) and inoculation of eye and nares by course spray of a 10^8 cfu culture of *E. coli* at 1 wk of age. Controls were neither stressed nor inoculated. Birds were bled and necropsied at 3 wk of age.

Table 1. Intermittent cold stress schedule for Experiment 3.

Age of bird (Days)	Duration of cold stress (Hours)	Temperature [1] ^{O}C
6	1	15.1 ± 2.2
7	2	13.3 ± 2.0
9	3	13.0 ± 1.6
11	7	13.1 ± 1.7
19	8	13.2 ± 1.0

[1]Mean value of temperature at beginning and end of cold stress ± SEM

Results

Experiment 1. Water supplementation of turkeys with vitamin D_3, significantly decreased mortality, disease incidence, and bacterial isolation from the liver due to Dex treatment and *E. coli* challenge (Figure 1). Challenged birds supplemented with vitamin D_3 had significantly improved body weight. Non challenged control birds supplemented with D_3 weighed an average of 547 g more than non-supplemented birds at 12 weeks of age, but this improvement was not significant (Figure 2). The heterophil/lymphocyte ratio was significantly decreased by vitamin D_3 treatment indicating modulation of the stress response (Figure 3).

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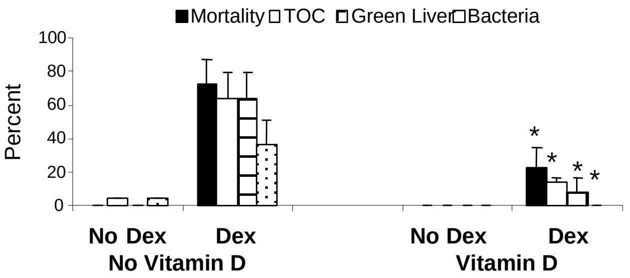

Figure 1. Effect of vitamin D_3 supplementation on mortality, incidence of turkey osteomyelitis complex (TOC) and green-liver, and bacterial isolation from the liver in a dexamethasone challenge model. *Indicates significant difference in Vitamin D supplemented birds as compared to non-supplemented ($P < 0.003$).

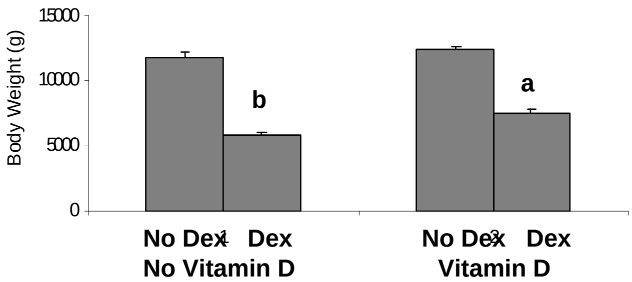

Figure 2. Effect of vitamin D_3 supplementation on body weight of dexamethasone treated turkeys. [a,b] Means with different superscripts are significantly different ($P = 0.0005$).

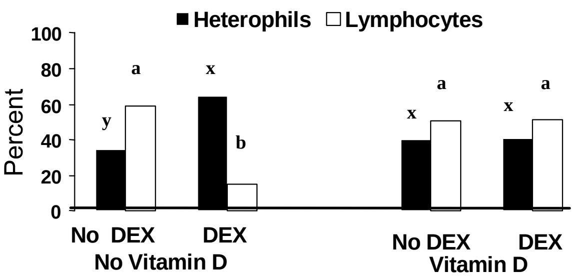

Figure 3. Effect of vitamin D_3 supplementation on the heterophil:lymphocyte ratio, a recognized measure of the response to stress. [a,b] Percent lymphocyte means with different superscripts are significantly different ($P = 0.0001$. [x,y] Percent heterophil means with different superscripts are significantly different ($P = 0.006$).

Experiment 2. Both 10 and 20 g/tonne Immustim® increased body weight of unchallenged turkeys (Figure 4) and 20 g/tonne protected them from the weight loss associated with *E. coli* respiratory challenge. There was a tendency for improved feed conversion efficiency (FC) of unchallenged birds and FC of *E. coli* challenged birds was improved by 20, 40, and 80 g/tonne.

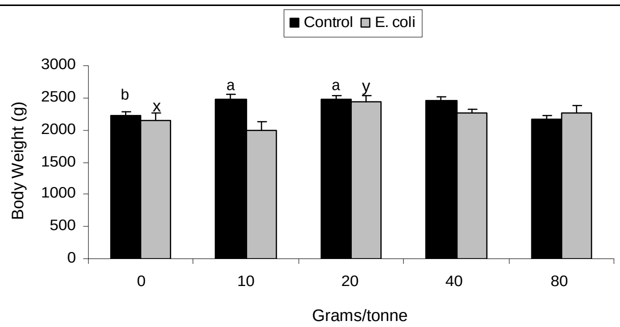

Figure 4. Body weight of unchallenged controls and *Escherichia coli* challenged 7-wk-old turkeys fed 0, 10, 20, 40, or 80 g/tonne Immustim. [a,b] Mean body weight of non-challenged birds with different superscripts are significantly different. [x,y] Mean body weight of *E. coli* challenged birds with different superscripts are significantly different.

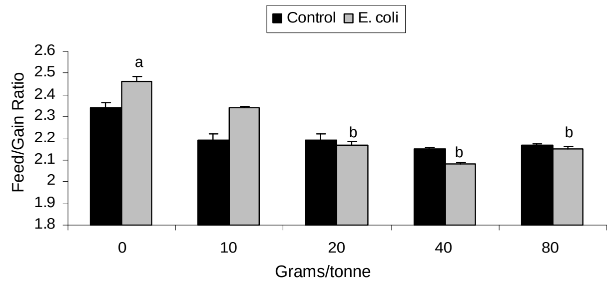

Figure 5. Feed/gain ratio of unchallenged controls and *Escherichia coli* challenged 7-wk-old turkeys fed 0, 10, 20, 40, or 80 g/tonne Immustim. [a,b] Means with different superscripts are significantly different.

Experiment 3. Pre-challenge (week 1) body weight was increased by Alphamune™ (Figure 6). Challenged, control fed birds had significantly decreased week 3 body weight compared to non-challenged controls and body weight of challenged poults was protected by both levels of supplementation (Figure 7). Feed/gain ratio was increased by cold stress ($P = 0.004$) and this increase was prevented by both levels of Alphamune™ (Figure 8).

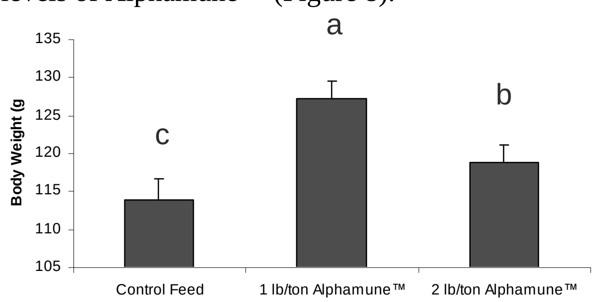

Figure 6. Pre-challenge body weight (week 1) of poults was increased more by 1lb/ton (504 g/tonne)($P<0.0001$) than by 2 lb/ton (1008 g/tonne) Alphamune™ ($P = 0.05$). [abc] Means with different superscripts are significantly different.

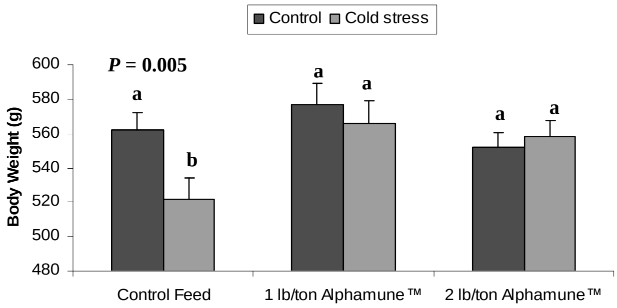

Figure 7. Week 3 body weight of poults was decreased by cold stress (*P* = 0.005) and this decrease was prevented by both levels of Alphamune™. [a,b] Means with different superscripts are significantly different.

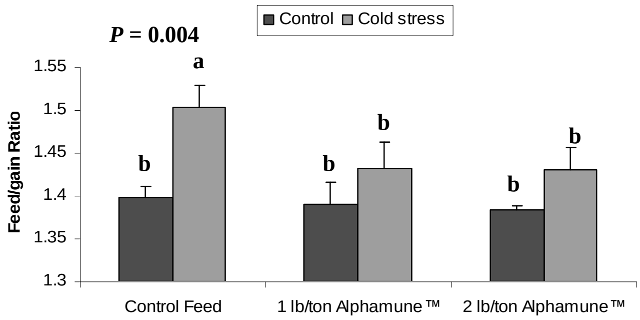

Figure 8. Feed/gain ratio of poults was increased by cold stress (*P* = 0.004) and this increase was prevented by both levels of Alphamune™. [a,b] Means with different superscripts are significantly different.

Discussion

There are a number of potential immunomodulators that may serve as alternatives to antibiotics for both growth promotion and disease resistance in poultry production. β-glucans are polymers of glucose that can be derived from the cell walls of yeast, bacteria, fungi, and cereals such as oats, barley, and rye. Each type of β-glucan has a unique structure in which glucose molecules are linked together in different ways, giving them different physical properties. Variations in molecular weight, degree of branching, conformation, and intermolecular associations can affect their biological activity (Bohn and BeMiller 1995). There is an extensive literature describing the immunomodulating effects of β-glucan in mammals, with most reporting an increase in functional activity of macrophages and neutrophils (Reynolds et al. 1980, Yun et al. 2003).

One of these molecules, the ß-1,3/1,6-glucan from the cell wall of *Saccharomyces cerevisiae*, is recognized as foreign by the immune systems of mammals, fish, and birds and has been shown to be protective in a number of disease challenge studies (Williams and Di Luzio 1979, Reynolds et al. 1980). β-glucans have been shown to improve immune function by activating macrophages, and taken orally, they have been shown to indirectly stimulate innate immunity in the respiratory system of mice by activating macrophages in the Peyer's patches of the gut (Sakurai et al. 1992). β-glucans are generally recognized as safe (GRAS) by the FDA for use as food and feed additives and are widely marketed as immunomodulators in the human nutraceutical market. In addition to the data reported here, in which 20 g/tonne of a purified β-glucan product was most protective in an *E. coli* challenge, turkey field studies (Bahl and Sorgente 2002), a controlled chicken battery study (Huff et al. 2006), and challenge of young chicks with *Salmonella enteritidis* (Lowry et al. 2005) have also suggested that ß-1,3/1,6-glucan may be useful as an alternative to growth promoting antibiotics in poultry production, however these studies also suggest that the dosage is extremely important.

Other potential immunomodulators that may serve as alternatives to antibiotics for both growth promotion and disease resistance in poultry production are less purified yeast products that include both the immunostimulating β-glucan molecules as well as the mannanoligosaccharide components of yeast. Brewer's yeast extracts, which are by-products of beer manufacturing, have been added to animal feeds for years for their nutritional content. Brewers dried yeast has been used as a source of both mannanoligosaccharides (MOS) and β-glucans by a number of companies providing antibiotic-replacement products for animal production. Whole yeast or yeast cell walls have been shown to improve growth of both turkey poults (Bradley et al. 1994) and broiler chicks (Zhang et al. 2005). Our studies with Alphamune™, have suggested that this type of product may modulate the stress response, but that as with β-glucan, the effective dose is dependant on both environmental and genetic factors (Huff et al. 2007).

Vitamin D_3 is considered to be a pro-hormone that is involved in homeostasis of diverse biological systems (DeLuca and Zierold 1998, De Luca 2008). Recently, vitamin D_3 has become recognized for a major role in resistance to bacterial disease due to its function as a defensin and its role in regulation of immunity (Adams et al. 2007, Bikle 2008).

Our studies with vitamin D_3 suggest that water supplementation of repeatedly stressed turkeys with vitamin D_3 increases resistance to bacterial infection and improves body weight gains (Huff et al. 2000a). However, in another study the effects of feed supplementation with the biologically active vitamin D metabolites, 1,25-dihydroxyvitamin D_3 and 25-hydroxyvitamin D_3, were more complex due to toxicity problems and were not as effective at increasing disease resistance in our challenge model (Huff et al. 2002a).

In summary, the results of these three animal studies indicate that there are potential uses for both yeast extract products and vitamin D supplementation in mitigating the immunosuppressive effects of chronic stress in turkey production. However, the dosage in relation to the level of stress is critical, there is a high degree of individual variability in response, and further research is needed to make widespread supplementation practical. By extrapolation, these products may be useful for improving the stress response in humans, but care should be taken to recognize the impact of individual variability in dose-response.

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