

Effects of a dietary yeast extract on hematological parameters, heterophil function, and bacterial clearance in turkey poult challenged with *Escherichia coli* and subjected to transport stress

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There is a need to develop nutritional methods for controlling pathogens in poultry production. A standardized yeast extract supplement, Alphamune™ (YE), was added to turkey poult diets. Male poults were challenged by air sac injection with 60 cfu of *E. coli* at 1 week of age. At 3 weeks of age challenged birds were subjected to transport stress and birds were bled and necropsied the following morning. Blood cell numbers and percentages and hematological parameters were determined. Oxidative burst activity of isolated heterophils was measured using stimulation with phorbol myristate acetate and a 2',7'-dichlorofluorescein diacetate (DCF-DA) assay. Data was analyzed using GLM and LS Means procedures of the SAS® program. The percentage of heterophils in peripheral blood was increased and their oxidative burst activity was stimulated by YE. Transport stress also increased oxidative burst and this increase was modulated by YE treatment. Serum levels of calcium, phosphorus, and triglycerides were decreased and uric acid levels, erythrocyte numbers, hemoglobin and hematocrit were increased by YE supplementation. Bacteria were isolated from the air sac and liver of a lower percentage of birds provided with YE. These results suggest that dietary YE has potential as a non-antibiotic alternative for decreasing bacterial pathogens in turkey production.

KEYWORDS: turkeys; yeast extract; Alphamune™; transport stress; heterophils

Introduction

Stress has negative effects on growth, feed conversion, and immune function in poultry (Siegel, 1995). For many years growth promoting and therapeutic antibiotics have been used to compensate for the high levels of stress that can be present in intensive animal production. While growth promoting antibiotics are thought to function mainly by changing the intestinal bacterial flora and affecting gut development, another mechanism by which they may improve production values is through their ability to modulate the inflammatory response to subclinical disease with the opportunistic pathogens that are present in the environment, such as *Escherichia coli*. The stresses of intensive poultry production can lead to changes in the immune response that make animals susceptible to these pathogens and thus lead to disease. Our research program, using an *E. coli* respiratory disease challenge model, has allowed us to study the effects of different stressors on disease and develop nutritional strategies for increasing both disease resistance and production values in turkeys and broiler chickens (Huff *et al.*, 2000, 2004, 2006).

The primary method of controlling *E. coli* infections in poultry is through the use of therapeutic antibiotics (Barnes, 2003). Since the 1950's sub-therapeutic levels of antibiotics have been used to improve production values, particularly feed conversion efficiency, presumably by modifying gut

bacterial ecology and development (Dibner and Richards, 2005). Sub-therapeutic antibiotics are also credited with decreasing morbidity and mortality from both clinical and subclinical infections with opportunistic pathogens such as *E. coli* (Gersema and Helling, 1986) and have allowed the development of increasingly intensive confinement animal production. However, the development of antibiotic resistance by pathogenic bacteria has led to a worldwide consideration of limiting the usage of antibiotics in animal agriculture. It is therefore essential to develop alternative ways of dealing with bacterial challenges in commercial poultry production.

Yeast cell walls are potential immunomodulators that may serve as alternatives to antibiotics for both growth promotion and disease resistance in poultry production. Brewer's yeast (*Saccharomyces cerevisiae*) extracts, which are by products of beer manufacturing, have been added to animal feeds for many years for their nutritional content. Brewers dried yeast has been used as a source of both mannan-oligosaccharides (MOS) and β -1,3/1,6-glucan by a number of companies providing antibiotic-replacement products for animal production. Whole yeast or yeast cell wall components have been shown to improve growth of both turkey poult (Bradley *et al.*, 1994) and broiler chicks (Zhang *et al.* 2005, Huff *et al.*, 2006). Both β -1,3/1,6-glucan and MOS are generally recognized as safe (GRAS) by the FDA for use as food and feed additives. Alphamune™ (Alpharma Animal Health) is a yeast extract feed additive that combines both the immunomodulatory properties of a standardized level of (1,3)/(1,6) β -glucans with the performance enhancement of MOS.

Materials and methods

Day-of hatch poult were obtained from a commercial hatchery and placed in battery brooders. Poults were fed an unmedicated turkey starter diet or the same diet supplemented with 1 lb/ton (@ 500 g/ton) or 2 lb/ton (@ 1000 g/ton) of Alphamune™ yeast extract (YE). There were 3 randomized pens with 10 birds/pen in each treatment group. Poults were challenged by air sac injection with 60 cfu of *E. coli* at 1 week of age. At 3 weeks of age these challenged birds were also subjected to transport stress. Birds were placed in coops and driven for 3 hours, then held in the same coops for 9 hours, giving a total of 12 hours of containment without feed or water. Treatment controls were neither stressed nor inoculated. Birds were returned to their original pens and provided feed and water. The next morning nine birds from each experimental group (3 birds/pen) were bled by cardiac puncture and all birds were necropsied.

Erythrocyte numbers, hemoglobin, hematocrit, and the numbers and proportions of white blood cells were measured in whole blood using a Cell-Dyn 3500 blood analysis system (Abbott Diagnostics, Abbott Park, IL) which employs both electronic impedance and laser light scattering and has been standardized for analysis of turkey blood. Serum was collected and stored at -20°C until assayed. Clinical chemistry analysis of serum levels of calcium, phosphorus, triglycerides, and uric acid were measured using an Express Plus automated clinical chemistry analyzer (Ciba-Corning Diagnostics Corp., Medfield, MA).

Heterophils from control fed birds and from those fed 2 lb/ton YE were isolated using a Histopaque gradient, and the oxidative activity of heterophils was measured using stimulation with phorbol myristate acetate (PMA) and a 2',7'-dichlorofluorescein diacetate (DCF-DA) assay as previously described (Farnell *et al.*, 2006).

Transport swabs were used to culture air sac and liver of each bird and were immediately taken to the laboratory where they were plated on MacConkey agar. Representative lactose-negative colonies were identified using API 20-E test kits (Bio-Mérieux Vitek Inc, Hazelwood, MO) and were compared to the challenge strain. Data were analyzed by pen as a 3 x 2 factorial arrangement (3 feed treatments x 2 challenges) using the General Linear Models Procedure of SAS software for analysis of variance. Means were separated using Duncan's Multiple Range Test and the Least Square Means Procedure with pen as the experimental unit (SAS, 1999).

Results and discussion

The percentage of heterophils in whole blood was significantly increased by both 1 and 2 lb/ton YE supplementation ($P=0.02$). There was a significant interaction affecting heterophil percentage between YE supplementation and transport stress ($P=0.02$). Heterophil oxidative burst was increased by both transport stress ($P < 0.0001$) and 2 lb/ton YE supplementation ($P = 0.001$), and YE appeared to modulate the effects of transport stress on oxidative burst activity (Figure 1).

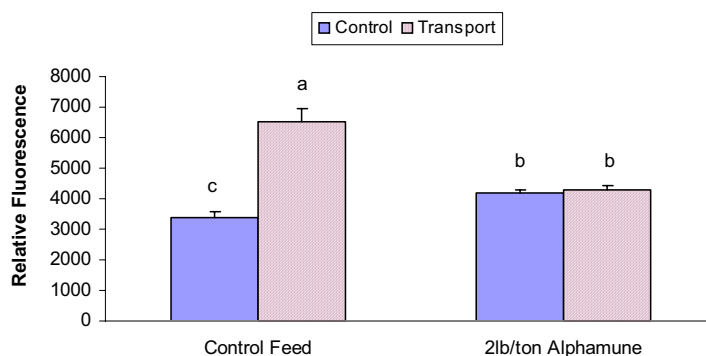


Figure 1. Effects of transport stress and 2 lb/ton Alphamune supplementation on oxidative burst activity of heterophils. Data is presented as mean and SEM relative fluorescence of PMA stimulated cells. Means with no common superscripts are significantly different ($P < 0.05$).

Serum levels of calcium, phosphorus, and triglycerides were decreased by YE supplementation. Uric acid levels were increased by both transport stress and the 2 lb/ton YE treatment. Erythrocyte numbers, hemoglobin and hematocrit were increased by 2 lb/ton YE supplementation.

The challenge strain of *E. coli* was isolated from the livers of 8.5% of birds fed the control diet, 3.4% of birds fed 2 lb/ton YE and 0% of birds fed 1 lb/ton YE ($P=0.05$). Air sac isolation of the challenge strain was positive in 5% of the control fed birds, 1.7% of birds fed 2 lb/ton YE and 0% of birds fed 1 lb/ton YE. While these differences were not significant, isolation of generic *E. coli* from the air sac was positive in 10% of birds fed the control feed and 1.7% of birds fed either 1lb/ton or 2 lb/ton YE ($P = 0.03$).

Systemic infection with *E. coli* (colibacillosis) is an important poultry disease and is the most frequent cause of airsacculitis leading to condemnation (Barnes *et al.*, 2003). While airsacculitis has a complex etiology, host susceptibility is thought to be more important than bacterial virulence, and abnormal stress is associated with infection (Barnes *et al.*, 2003). One of the stressors that is associated with an increased incidence of *E. coli* respiratory disease and subsequent joint and bone infection is moving turkeys from to larger houses in a 2 or 3-stage grow-out system (Huff *et al.*, 2000). The transportation of turkeys to the processing plant, when accompanied by long holding times due to delays in the inspection procedure, is also thought to lead to an increase in lesions of colibacillosis (Huff *et al.*, 2000).

In this experimental transport stress challenge YE was shown to increase heterophil percentages and oxidative burst activity and these changes were correlated with a decrease in the isolation of *E. coli* from both airsac and liver. In addition, YE modulated the dramatic increase in oxidative burst due to transport stress. The heterophil is a highly phagocytic granulated cell capable of antimicrobial activity and is analogous, though not identical, to the mammalian neutrophil (Harmon, 1998). While activation of heterophils is an important component of the innate immune response, these cells are also responsible for inflammatory tissue damage. In mammals, the rate of neutrophil production can increase 10-fold during stress and infection (Smith, 1994). Human patients with infection have been show to have a subpopulation of neutrophils with an enhanced oxidative burst and males subjected to moderate exercise had increased percentages of neutrophils that were highly responsive to PMA (Smith, 1994). In this study, transport stress dramatically increased the oxidative burst activity of

PMA stimulated cells. YE supplementation moderately increased oxidative burst of non-challenged controls but infection and transport stress did not result in a further increase.

Uric acid is a major antioxidant in birds and is used as a biomarker for physiological processes such as oxidative stress and tubular function (Hartman et al., 2006). In this study both the transport stress challenge and 2 lb/ton YE supplementation increased serum uric acid levels. In addition, changes were seen in other hematological and physiological indices, suggesting a need for further study of the effects of yeast extracts on bird physiology.

Stress has variable effects on the immune system and can both enhance and suppress responses depending on the type and degree of stress and individual variation in the host response (Siegel, 1995). These data suggest that yeast extract supplementation may help to modulate the oxidative stress response in turkeys challenged with *E. coli* and subjected to transport stress.

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