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# Purified β-glucan as an abiotic feed additive up-regulates the innate immune response in immature chickens against *Salmonella enterica* serovar *Enteritidis*

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**Abstract**

Functionally, the innate immune system of immature chickens is inefficient during the first week posthatch. This immunological inefficiency enables pathogens such as *Salmonella enterica* serovar *Enteritidis* (SE) to invade and colonize the visceral organs of immature chickens. The objective of this study was to evaluate the effect of purified β-glucan as an immunomodulator of the innate immune response. β-glucan, as a feed additive, significantly provided protection against SE organ invasion in young chickens ($P<0.05$). The functional efficiency of heterophils isolated from neonatal chickens fed a β-glucan ration was significantly ($P<0.05$) up-regulated when compared to heterophils isolated from chickens fed a control ration as determined with an array of functional assays. Phagocytosis, bactericidal killing, and oxidative burst were significantly increased in heterophils isolated from chickens fed the purified β-glucan ration ($P<0.05$). To our knowledge, this is the first report of a purified β-glucan feed additive significantly decreasing the incidence of SE organ invasion in immature chickens and up-regulating the functional abilities of heterophils isolated from immature chickens against an invading pathogen, SE.

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*Keywords:* *Salmonella enteritidis*; Heterophil; β-glucan; Chickens; Organ invasion; Innate immunity

## 1. Introduction

Young poultry are most susceptible to invasive pathogens during the first week posthatch (Pomeroy and Nagaraja, 1988). Salmonellosis is well documented as a debilitating disease in young poultry

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(Pomeroy and Nagaraja, 1988). A functionally inefficient innate immune response in chickens during the first week posthatch has been previously demonstrated in our laboratory and by other investigators (Kogut et al., 1994; Lowry et al., 1997; Genovese et al., 1998; Jones et al., 2001). Consequently, researchers, producers, and veterinarians are constantly developing management strategies to protect commercial and private poultry flocks against invading pathogens, i.e., *Salmonella* species.

Paratyphoid *Salmonella* infections continue to affect the poultry industry and contribute to human food-borne disease in both the United States and Europe despite global implementation of programs encompassing measures of biosecurity, vaccination, sanitation, and medication (Lister, 1988; O'Brien, 1988; St. Louis, 1988; Bartlett et al., 1989; Gast and Beard, 1993). The development of practical methods for reducing paratyphoid *Salmonella* infections and contamination in poultry through public education and to promote safe food handling are needed and have become issues of increasing importance. Certain groups within the United States and the European Union propose elimination of antibiotics used as growth promoters from the feed rations of animals raised for food production, i.e., poultry, pork, beef, and resulting dairy products, such as milk products and eggs, would be affected as well (Fox, 2003; McDonald's, 2003). When table eggs are not handled properly, eaten raw or under cooked, *Salmonella enterica* serovar *Enteritidis* (SE) is the primary pathogen responsible for food poisoning in humans (St. Louis, 1988; Bartlett et al., 1989; Tauxe, 1991; Gast and Beard, 1993). SE contamination commonly occurs from the hen to the egg (Chiodini, 1982; Wigley et al., 2001). To reduce the incidence of egg and meat contamination, the prevalence of invading pathogens such as SE must be decreased or preferably eradicated in layer and meat-producing flocks.

Research efforts in our laboratory have focused on developing cytokine-mediated immunoprophylactic strategies (Bischoff et al., 2001; Kogut et al., 2002; Crippen et al., 2003a,b) and selective genetics (Swaggerty et al., 2003a,b) that prevent or control intestinal *Salmonella* organ colonization in poultry. A complementary strategy to those previously mentioned would be an abiotic feed additive to up-regulate the innate immune response in immature chickens.

β-glucan, in many forms, has been well documented in the literature as an in vitro immunomodulator of mammalian macrophages and neutrophils (Kokoshis et al., 1978; Williams, 1997; McLeish et al., 1998; Kataoka et al., 2002). The major objectives of our study were twofold: (1) does the addition of purified β-glucan to a feed ration without the benefit of antibiotics as growth promoters decrease the incidence of SE organ invasion in immature chickens; and (2) does a purified β-glucan feed ration stimulate a heterophil-mediated innate immune responses in immature chickens.

To our knowledge, this is the first documented report describing purified β-glucan fed to chickens the first week posthatch significantly decreasing the incidence of visceral organ invasion against an invading enteropathogen, such as SE, and enhancing the functional efficacy of immature heterophils against an invasive enteropathogen.

## 2. Materials and methods

### 2.1. SE organ invasion

#### 2.1.1. Bacterial challenge organism

An isolate of *S. enterica* serovar *Enteritidis* (SE) was obtained from NVSL, Ames, IA (ID# 9711771, PT 24). The isolate was selected for resistance to carbenicillin and novobiocin (CN) and was maintained in tryptic soy broth or on tryptic soy agar at 4 °C. Brilliant Green Agar (BGA), a selective culture media for *Salmonella*, was used to culture the resistant isolate in experimental studies and contained 100 μg/ml carbenicillin and 25 μg/ml novobiocin (CN) to inhibit growth of other bacteria (BGA+CN). Inocula for challenge was prepared from 18 to 24 h tryptic soy broth (TSB)+CN cultures maintained at 41 °C and diluted in sterile PBS (pH 7.2). A stock solution ($1\times10^9$ cfu/ml) was prepared, and bacterial concentration was determined spectrophotometrically using a standard curve at a reference wavelength of $\lambda$=625 nm.

#### 2.1.2. Experimental animals

One-day-old White Leghorn roosters obtained from a commercial hatchery were transported to our laboratory and maintained in a biohazard isolation unit at the USDA in College Station, TX. Each

experimental group of animals was maintained in a separate isolation room. Each isolation room contained a floor pen with pine-shaving litter to house the chickens. Animals were provided ad libitum access to water and a balanced unmedicated corn and soybean meal based diet. All feed rations contained the levels of critical nutrients recommended by the National Research Council (1994). *Salmonella* were not detected in the feed or from paper tray liners. All feed was provided by ImmuDyne, 11200 Wilcrest Green Dr., Houston, TX 77042.

#### 2.1.3. Experimental design

One-day-old White Leghorn roosters were randomly distributed into three experimental groups: Group 1 contained 30 roosters housed in a separate isolation room and fed the β-glucan-supplemented ration throughout the experiment (treatment, no SE challenge, negative control). Group 2 contained 30 roosters housed in a separate isolation room and was fed the β-glucan ration throughout the experiment (treatment, SE challenge). Group 3 contained 30 roosters housed in a separate isolation room and was fed the control ration (without β-glucan) throughout the experiment (no treatment, SE challenge-positive control). Three days posthatch, all chickens in Groups 2 and 3 were orally challenged with $5\times10^7$ cfu/ml SE+CN and maintained on feed throughout the experiment. Four days post hatch (24 h post SE+CN challenge), all experimental animals were euthanized. From each chicken, the liver and spleen were aseptically removed, minced, combined in 50 mls tetrathionate broth for enrichment, and incubated overnight at 41 °C. From each enrichment culture, 10 μl was streaked onto a BGA+CN plate for isolation of SE and incubated for 24 h at 41 °C. Subsequently, BGA+CN plates were examined for the presence of typical nonlactose fermenting CN resistant *Salmonella* colonies. Colonies were randomly selected from each plate and confirmed SE-positive or -negative with a commercial antisera (Andrews et al., 1978). Three experimental trials were conducted on three different dates.

#### 2.1.4. Statistical analysis

The data from each experimental group was pooled from three separate trials for statistical analysis. Statistical differences between treatment groups were determined by ANOVA ($P<0.001$). Means were further separated for significance with an all pairwise multiple comparison procedure (Tukey test, $P<0.05$; SigmaStat, 1994).

### 2.2. Heterophil functional assays

#### 2.2.1. Experimental animals

One-day-old White Leghorn roosters were maintained, fed, and cared for as previously mentioned in Section 2.1.2. On the fourth day, all animals were euthanized for peripheral blood collection and heterophil isolation.

#### 2.2.2. Isolation of peripheral blood heterophils

Avian heterophils were isolated from peripheral blood of 4-day-old White Leghorn chickens as previously described (Kogut et al., 1994, 1998). Disodium ethylenediaminetetraacetic acid (EDTA)-anticoagulated blood was mixed with 1% methylcellulose at a 1.5:1 ratio and centrifuged at $25\times g$ for 30 min. The serum and buffy coat layers were retained and suspended in $Ca^+$, $Mg^+$-free Hanks' balanced salt solution (HBSS, 1:1). This suspension was layered over a discontinuous Ficoll-Hypaque gradient (specific gravity 1.077 over specific gravity 1.119). The gradient was then centrifuged at $250\times g$ for 60 min. After centrifugation, the 1.077/1.119 interfaces and 1.119 fractions containing the heterophils were collected and washed in RPMI 1640 medium ($2000\times g$, 15 min, 4 °C) and resuspended in fresh RPMI 1640. Cell viability was determined by trypan blue exclusion. The purity of the heterophil suspension was assessed by microscopic examination of cytospin-stained smears. Heterophil preparations obtained by this method were typically >95% pure and >95% viable. Heterophils were kept on ice until diluted to the desired working concentration for each functional assay.

#### 2.2.3. Live bacteria

Live SE with out CN was prepared as previously described in Section 2.1.1, kept on ice and diluted to the desired working concentration for use in functional assays.

#### 2.2.4. Opsonization of Salmonella enteritidis

Normal chicken serum (NCS) was used as a source of complement for opsonization of SE as previously described (Kogut et al., 1995). Live SE ($10^8$ cfu/ml)

was suspended in pooled NCS (1 part NCS: 4 parts live SE), opsonized for 30 min at 39 °C on a rotary shaker, washed twice with $Ca^{+}$, $Mg^{+}$-free HBSS, and stored at 4 °C in HBSS until used (OpSE=opsonized SE).

*2.2.5. Phagocytosis of live SE*

Phagocytosis of SE by heterophils isolated from each experimental group was determined by modifying a previously described procedure (Wigley et al., 2002). Heterophils were isolated from peripheral blood and adjusted to a working concentration of $5\times10^6$ cells/ml. Live SE was prepared as described in Section 2.2.3 to a working concentration of $5\times10^7$ cfu/ml. A ratio of 10 bacteria (SE):1 heterophil was maintained in each experiment. To achieve maximum contact between bacteria and cell, the 10:1 (SE/heterophil) mixture was pipetted into a sterile 15-ml conical centrifuge tube and centrifuged ($2000\times g$ for 15 min at 4 °C). Upon completion of centrifugation, all samples were incubated at 39 °C+5% $CO_2$ for 1 h. Following the 1 h incubation, samples were submerged in an ice bath for 15 min to stop phagocytosis. Samples were pelleted by centrifugation ($2000\times g$ for 15 min at 4 °C), supernatants were decanted, cell/bacteria pellets were resuspended in ice-cold clear RPMI 1640 and washed ($2000\times g$ for 15 min at 4 °C). Upon completion of the wash, supernatants were decanted and cell/bacteria pellets were resuspended in 2 ml ice-cold gentamicin solution (100 μg/ml in clear RPMI 1640) and incubated 1 h on a rocker at 37 °C. The gentamicin incubation removes residual extracellular SE from the cell/bacteria preparation. Following the gentamicin incubation, all samples were pelleted via centrifugation ($2000\times g$ for 15 min at 4 °C), supernatants decanted and pellets resuspended in ice-cold clear RPMI 1640. Resuspended pellets were washed accordingly ($2000\times g$ for 15 min at 4 °C). The washing step was repeated twice (total of three washes ) for each cell/bacteria pellet. Upon completion of the third wash, supernatants were decanted, and cell pellets were resuspended to the original volume with ice-cold clear RPMI 1640. From each sample, three replicate cytospin smears were made and examined microscopically. Four separate experiments were performed on different dates and replicated in triplicate. Data were pooled for statistical analysis and presentation. 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)×(the average number of bacteria per ingesting heterophil)×100. Only bacteria contained within a defined vacuole were counted as being phagocytized by heterophils.

*2.2.6. Bactericidal assay*

Bactericidal activity of peripheral blood heterophils from each experimental group was evaluated as previously described (Stevens and Olsen, 1993; Kogut et al., 1995). Four separate experiments were conducted on different dates, and samples from all experimental groups were replicated in quadruplicate. Bactericidal activity of the test wells (bacteria+heterophils from each experimental group) was extrapolated from a standard curve, generated using known bacterial concentrations ($5\times10^5$ to $5\times10^4$ cfu). Samples within a plate were compared to a standard curve within that same plate to minimize variation on a day-to-day and plate-to-plate occurrence. Data were pooled for statistical analysis and presentation. The results are expressed as percent bactericidal activity.

*2.2.7. Degranulation assay*

Degranulation was monitored by quantifying the micromoles of β-D-glucuronidase released in the supernatants of heterophils as previously described (Dewald and Baggiolini, 1986; Terashima et al., 1996; Yu and Czupryniski, 1996; Kogut et al., 2001). Heterophils ($8\times10^6$ cells/ml) were treated with OpSE or RPMI 1640 for 1 h at 39 °C on a rotary shaker. The samples were immersed in ice for 10 min to stop the reaction. Cells were removed by centrifugation ($250\times g$ for 15 min at 4 °C), the supernatants collected and assayed for β-D-glucuronidase activity. Samples and standards (25 μl) were added to a black flat-bottom ELISA plate and incubated with 50 μl freshly prepared substrate (10 mM 4-methylumbelliferyl-β-D-glucuronide dissolved in 0.1 M sodium acetate solution containing 0.1% Triton X-100; pH 4.0) for 4 h at 41 °C. The reaction was stopped by the addition of 200 μl stop solution (0.05 M glycine, 5 mM EDTA; pH 10.4). Liberated 4-methylumbelliferone was measured on a *f* max fluorescence microplate reader (Molecular Devices, Sunnyvale, CA; excitation=355 nm, emission=460 nm) and converted using a standard curve of known concentrations. Data were pooled

for statistical analysis and presentation. The results are expressed as micromoles β-glucuronidase released into the cell supernatants.

*2.2.8. Oxidative burst assay*

Oxidative burst was quantified by luminol-dependent chemiluminescence (LDCL) as previously described (Merrill et al., 1996). Briefly, heterophils ($4\times10^6$ cells/ml) were incubated with either phorbol A-myristate 13-acetate (PMA; 2.0 μg/ml) or RPMI 1640 for 30 min at room temperature in the dark. Samples were inverted every 5 min to ensure sufficient mixing. Subsequently, 400 μl of each sample was pipetted into a polyethylene scintillation vial containing 5-amino-2-3-dihydro-1,4-phthalazinedione sodium salt (luminol; 500 μl of 0.01 M luminol in clear RPMI 1640). Samples were counted for 1 min/vial in an LKB A19 liquid scintillation counter (LKB Products, Turku, Finland) using the tritium channel and the incoincidence mode. Each sample was assayed in replicates of 10 vials/experiment and expressed as an average counts per minute (cpm). Four separate experiments were conducted on four different dates. Data were pooled for statistical analysis and presentation.

*2.2.9. Experimental design*

Immunomodulation of chicken heterophils by dietary β-glucan: day-of-hatch chickens were randomly divided into two experimental groups, 50 birds per group: (1) control feed ration containing no β-glucan; or (2) the control ration with β-glucan. Chickens had continual access to feed and water for 4 days before heterophils were collected from the peripheral blood. The anticoagulated blood from all chickens in each experimental group was pooled to eliminate day-to-day variation in assay procedures, and peripheral blood heterophils were isolated from each experimental group as described in Section 2.2.2. Each heterophil functional assay (phagocytosis, bactericidal killing, degranulation, and oxidative burst) was conducted on four different dates. Data from these replicated experiments were pooled for statistical analysis and presentation.

*2.2.10. Statistical analysis*

The data from each experimental group was pooled from four separate trials for statistical analysis. Statistical differences between treatment groups were determined by ANOVA ($P<0.001$). The means from the oxidative burst and degranulation assays were further separated for significance with an all pairwise multiple comparison procedure (Tukey test, $P<0.05$; SigmaStat, 1994). The means from the phagocytosis and bacterial killing assays were further separated for significance with *t*-test, $P<0.05$ (SigmaStat, 1994).

## 3. Results

*3.1. SE organ invasion*

The incidence of SE positive liver/spleen (L/S) enrichment cultures was significantly different ($P<0.05$) between chickens fed the β-glucan ration

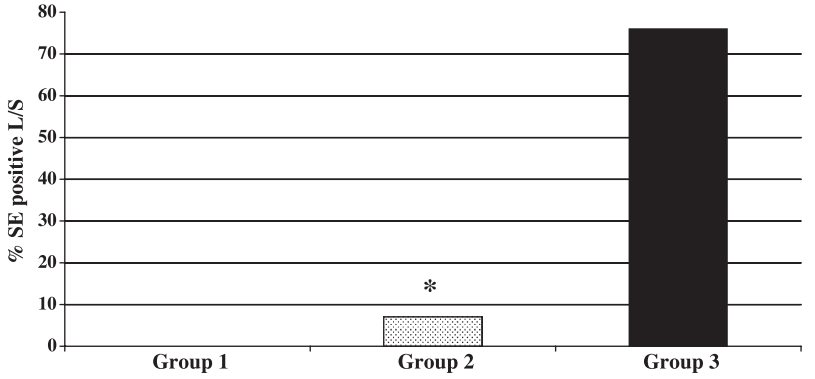


Fig. 1. Effects of dietary β-glucan on SE organ invasion in immature chickens. β-glucan fed as a nutritional supplement to neonatal chickens 3 days prior to SE challenge significantly reduced the incidence of SE organ invasion when compared to the incidence of SE organ invasion in chickens fed a control fed ration 3 days prior to SE challenge (*indicates statistically significant differences, $P<0.05$; SigmaStat, 1994).

Table 1
Effects of feeding β-glucan on chicken heterophil phagocytosis

| Treatments | Percent heterophils+SE | Mean #SE/ heterophil | Phagocytic index (PI) |
|---|---|---|---|
| Control feed | 38.54±0.05 | 4.38±1.08 | 175.54±44.92 |
| β-glucan feed | 78.84±0.03[a] | 8.20±0.76[a] | 644.10±57.07[a] |

Effects of feeding β-glucan on chicken heterophil phagocytosis. Heterophils isolated from chickens fed a control ration were compared to heterophils isolated from chickens fed a β-glucan-supplemented ration. Parameters analyzed for significance were as follows: percent heterophils containing SE, mean number of SE per heterophil, and phagocytic index (PI) (SigmaStat, 1994).

[a] Indicates statistically significant differences, $P<0.05$.

Table 2
Effects of dietary β-glucan on immature chicken heterophil degranulation

| Treatments | β-Glucuronidase released (μM)±S.E.M. |
|---|---|
| Control feed | 34.25±1.48[a] |
| Control feed+OpSE | 124.63±9.38[b] |
| β-Glucan feed | 37.03±1.38[a] |
| β-Glucan feed+OpSE | 134.42±12.21[b] |

Effects of dietary β-glucan on immature chicken heterophil degranulation. Heterophils isolated from chickens fed a control ration were compared to heterophils isolated from chickens fed a β-glucan-supplemented ration. There was no significant difference in the release of β-glucuronidase between heterophils isolated from chickens fed a control ration when compared to chickens fed a β-glucan-supplemented ration. The addition of opsonized bacteria did not significantly change the degranulation process (a,b: different letters within a column indicate a statistically significant difference, $P<0.05$; SigmaStat, 1994).

when compared to those fed the control ration for 3 days prior to challenge (Fig. 1). Experimental Groups 1 and 2 were both fed the β-glucan ration. Group 1 did not receive the SE challenge, Group 2 received the SE challenge, and no significant differences between experimental Groups 1 and 2 were determined, supporting our hypothesis that a β-glucan ration would decrease the incidence of SE organ invasion. Groups 1 and 2 (0% and 7% SE-positive L/S cultures, respectively) were both significantly lower ($P<0.05$) when compared to Group 3 (76%), the SE challenge group which received the control ration, further supporting our hypothesis that β-glucan as an abiotic feed additive would reduce the incidence of SE organ invasion in immature chickens (Fig. 1).

### 3.2. Heterophil functional assays

#### 3.2.1. Phagocytosis of live SE

β-glucan as an abiotic feed additive significantly increased the ability of immature chicken heterophils to phagocytize live bacteria when compared to heterophils isolated from chickens fed a control ration (Table 1). All parameters statistically analyzed were significantly greater in heterophils isolated from chickens fed the β-glucan ration when compared to heterophils isolated from chickens fed a control ration (Table 1). Heterophils isolated from chickens fed a β-glucan ration contained 40% more SE than heterophils isolated from chickens fed a control ration (Table 1). The mean number of SE per heterophil was two times greater in the β-glucan-supplemented group when compared to heterophils isolated from chickens fed the control ration (Table 1). The PI for heterophils isolated from chickens fed the β-glucan ration was significantly greater, 644.10, when compared to the PI for heterophils isolated from chickens fed the control ration, 175.53 (Table 1).

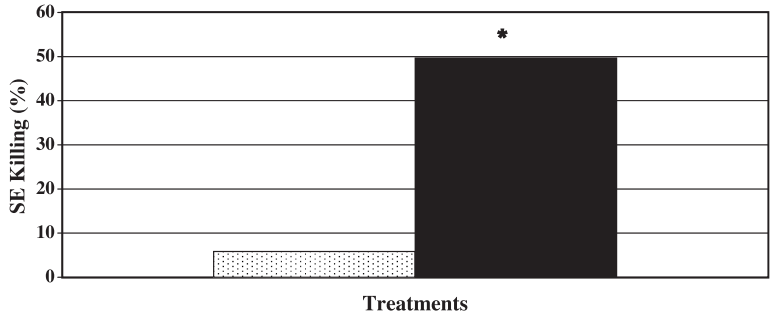


Fig. 2. β-glucan as a feed additive stimulates bactericidal killing in immature chicken heterophils. Heterophils isolated from chickens fed a control ration were compared to heterophils isolated from chickens fed a β-glucan ration. The percentage of SE killed by heterophils was analyzed for significance (*indicates statistically significant differences $P<0.05$). ▒ control feed, ▌β-glucan feed (SigmaStat, 1994).

*3.2.2. Bactericidal assay*

Percent killing of SE by heterophils: heterophils isolated from chickens fed the β-glucan ration not only phagocytized SE more efficiently than heterophils isolated from chickens fed the control ration, heterophils isolated from the chickens fed the β-glucan ration also killed SE more efficiently than those isolated from chickens fed a control ration (49.7% killing β-glucan ration and 5.83% killing control ration; Fig. 2).

*3.2.3. Degranulation*

Quantification of β-glucuronidase, a primary bactericidal enzyme released by the intracellular granules located in the cytoplasm of heterophils was compared in heterophils isolated from chickens fed a control ration to heterophils isolated from chickens fed a β-glucan ration. No statistically significant differences were determined in the release of β-glucuronidase, indicating degranulation is not the mechanism by which β-glucan modulates an innate immune response against OpSE (Table 2).

*3.2.4. Oxidative burst*

LDCL was used to determine a difference in the functional ability of PMA-stimulated heterophils isolated from chickens fed a control ration compared to heterophils isolated from animals fed a β-glucan-supplemented ration to produce an oxidative burst (Table 3). Heterophils isolated from chickens fed a β-glucan-supplemented ration and stimulated with PMA produced a significantly greater oxidative burst when compared to heterophils stimulated with PMA isolated from chickens fed a control ration (Table 3). There was no statistical difference between heterophils (not stimulated with PMA) isolated from chickens fed a control ration and those fed a β-glucan ration, indicating β-glucan is a priming agent for heterophils aiding in cellular efficacy to phagocytize and kill invading pathogens, such as SE.

Table 3
Effects of dietary β-glucan on immature chicken heterophil oxidative burst

| Treatments | cpm±S.E.M. ($10^6$) |
|---|---|
| Control feed | $0.60 \pm 0.03^{a}$ |
| Control feed+PMA | $5.09 \pm 0.26^{b}$ |
| β-Glucan feed | $0.89 \pm 0.07^{a}$ |
| β-Glucan feed+PMA | $10.03 \pm 1.05^{c}$ |

Effects of dietary β-glucan on immature chicken heterophil oxidative burst. Heterophils isolated from chickens fed a control ration were compared to heterophils isolated from chickens fed a β-glucan-supplemented ration. Control heterophils from each group were compared for significance as were heterophils stimulated with PMA. Oxidative burst was measured by LDCL. Heterophils isolated from chickens fed the β-glucan-supplemented ration stimulated with PMA released more oxygen free radicals than did PMA-stimulated heterophils isolated from chickens fed a control ration (a,b,c: different letters within a column indicate a statistically significant difference, $P<0.05$; SigmaStat, 1994).

## 4. Discussion

The immunostimulatory properties of β-glucan (in various forms) have been reported by other investigators in a variety of species, poultry, beef steers, salmon, and mice (Terashima et al., 1996; Estrada et al., 1999; Acevedo et al., 2001; Lee et al., 2003; Paulsen et al., 2003). Serum opsonized zymosan A, a complex form of β-glucan, has been shown to upregulate the exocytosis of arginine-specific ADP-ribosyltransferase and regulate cellular events, such as attachment, migration, and phagocytosis of heterophils isolated from market age broilers during inflammation (Terashima et al., 1996). Plasma lysozyme activity was increased by both LPS and β-glucan, and the lysozyme gene was reported to be transcribed in the heart, head kidney, spleen, liver, and intestine, and accumulation of transcript was demonstrated in response to both stimulants in all organs following intraperitoneal (i.p.) injection of LPS and β-glucan in Atlantic salmon (*Salmo salar* L.; Paulsen et al., 2003). Intracellular lysozyme activity was also detected in the aforementioned organs of the Atlantic salmon and from isolated blood PMNs and lymphocytes (Paulsen et al., 2003). T lymphocytes were stimulated (delayed hypersensitivity) when an oral and intraperitoneal β-glucan preparation were administered to 9-day-old chicks, and immunocompetence was determined by calculating the quotient of the bursa of Fabricius 24 h, 7, 14, and 21 day post-β-glucan administration (Acevedo et al., 2001). In vitro, peritoneal macrophages isolated from mannose receptor-deficient mice (MR−/−) were equally capable of *C. albicans* uptake, and phagocytosis was blocked with β-glucan, indicating that MR is not required for

the normal host defense during disseminated candidiasis or for the phagocytosis of *C. albicans*, and a β-glucan receptor may be required for phagocytosis of *C. alibicans* (Lee et al., 2003). β-glucan isolated from oats significantly restored various specific and non-specific immune responses when fed to beef steers previously immunosuppressed with dexamethasone (Estrada et al., 1999). Based on these results (Terashima et al., 1996; Estrada et al., 1999; Acevedo et al., 2001; Lee et al., 2003; Paulsen et al., 2003), we hypothesized purified dietary β-glucan may provide immunostimulatory properties necessary to reduce the incidence of SE in neonatal chickens.

Traditional management of infectious diseases in poultry has depended on the use of either broad-spectrum antibiotics and/or vaccines (Nagaraja et al., 1991). However, the public outcry for the removal of antibiotics in animal feed may ultimately limit the future production and availability of antibiotics even as therapeutic agents to veterinarians and producers (Fox, 2003; McDonald's, 2003). Unfortunately, poultry are the most susceptible to invading pathogens during the first week posthatch when the innate immune system is functionally inefficient. The objective of this study was to demonstrate immunomodulation of the avian innate immune response with a purified dietary β-glucan additive up-regulating the functional efficacy of the heterophil and providing protection from SE organ invasion.

The results described in this report are based upon the hypothesis that purified β-glucan in the diet of immature chickens during the first week post hatch would significantly enhance the functional efficacy of the heterophil and elicit an innate immune response against an invading pathogen (SE). β-glucan significantly up-regulated heterophil efficacy to phagocytize and kill invading SE via a respiratory burst (Table 3). Our data indicate β-glucan is a priming agent for the innate immune response. Heterophils isolated from chickens fed a β-glucan ration, stimulated with PMA in vitro, exhibited a significant ($P<0.05$) increase in the quantity of oxygen free radical released from these phagocytes when compared to heterophils isolated from chickens fed a control diet (Table 3). Due to the fact there was no significant difference in the ability of the heterophil to enhance an oxidative burst until stimulated with PMA, indicating priming with β-glucan is most likely a receptor-mediated event (Brown and Gordon, 2001; Brown et al., 2002; Taylor et al., 2002). To our knowledge, this is the first report of purified β-glucan as a feed additive priming the innate immune response.

Recently, Brown and Gordon (2001) and Brown et al. (2002) isolated and characterized the dectin-1 receptor on the surface of mammalian macrophages and neutrophils, determining specificity for β-glucan. Identification and characterization of the dectin-1 receptor, specifically for β-glucan, indicated protection provided by β-glucan is a receptor-mediated event against invading enteropathogens, i.e., SE (Jamas et al., 1998; Brown and Gordon, 2001; Brown et al., 2002; Taylor et al., 2002). A more recent report demonstrates a β-glucan receptor may be necessary for the phagocytosis of fungal pathogens by murine peritoneal macrophages, and the previously suspected mannose receptor is not involved (Lee et al., 2003). Therefore, we speculate, a dectin-1-like receptor is present on the surface of the heterophil. The dectin-1-like receptor would mediate the binding of β-glucan through priming the phagocyte for stimulation with an inflammatory agonist. Further studies are currently under way to define and characterize a dectin-1-like receptor on the surface of the heterophil.

Heterophils isolated from chickens fed the β-glucan diet when compared to heterophils isolated from chickens fed a control diet indicated no difference in the ability of either group of heterophils to degranulate when stimulated with OpSE (Table 2). The degranulation data demonstrates SE is killed by phagocytes exhibiting a respiratory bust and other antimicrobial moieties located in the cytoplasm of the heterophil, not contained within the primary granules, and released by the heterophil during phagocytosis (Bischoff et al., 2001; Crippen et al., 2003a,b).

The questions posed in our hypothesis were answered: (1) priming of the immature immune system with purified β-glucan apparently enabled the liver and spleen to effectively and efficiently decrease the incidence of invasive SE 24 h post-challenge (Figs. 1. and 2) purified β-glucan successfully primed immature chicken heterophils to phagocytize and kill invasive SE. Bactericidal killing was accomplished by releasing oxygen free radicals

into the peripheral blood through an effective respiratory burst on behalf of the heterophil (Fig. 2; Tables 1–3). Reemphasizing, to our knowledge this is the first report of a purified β-glucan molecule fed to immature chickens significantly up-regulating innate immunity.

Further research is necessary to determine the mechanisms by which β-glucan significantly up-regulates the immature avian innate immune response. Identification and characterization of a dectin-1-like receptor will also be of significant importance to scientists and producers as well.

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