Bacillus subtilis-based direct-fed microbials augment macrophage function in broiler chickens

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ABSTRACT

The present study was conducted to evaluate the function of Bacillus subtilis-based direct-fed microbials (DFMs) on macrophage functions, i.e., nitric oxide (NO) production and phagocytosis in broiler chickens. DFMs used in this study were eight single strains designated as Bs2084, LSSAO1, 3AP4, Bs18, 15AP4, 22CP1, Bs27, and Bs278, and one multiple strain DFM product (Avicorr™) containing equal amount of Bs2084, LSSAO1 and 15AP4. NO concentrations were monitored in plasma and in the supernatants from the peripheral blood-derived monocytes (PBMC)-derived macrophages stimulated by either chicken recombinant interferon gamma (IFNγ) or lipopolysaccharide (LPS) from Escherichia coli or Salmonella typhi. In addition, phagocytosis of fluorescent beads or green fluorescent protein (GFP)-labeled Salmonella by macrophages was assayed. Plasma NO levels were significantly higher in groups given 3AP4 or Bs27 diets compared with the control group at days 7 and 14. NO production by PBMC-derived macrophage was augmented in DFM-fed birds, while splenic lymphocyte proliferation, intestinal intraepithelial (IEL) subpopulation, and cytokine mRNA levels in IELs were affected depending on the strains of DFMs used. We also found that dietary DFMs increased the resistance of chickens to experimental coccidiosis (Lee et al., 2007a,b, 2010c). In this study, we further examined the role of B. subtilis-based DFMs on macrophage functions, i.e., nitric oxide (NO) production and phagocytosis, two important innate immune functions of macrophages (Lillehoj and Li, 2004; Munoz-Fernandez et al., 1992; Stuehr and Marletta, 1985). Macrophage, a key component of host innate immunity, participates in host defense by secreting cytokines (e.g., IL1, IL12 and TNF) and nitric oxide (NO) that modulate inflammation and kill microbes.

1. Introduction

Direct-fed microbials (DFM), commonly known as probiotics, are live microorganisms that could confer a health benefit on the host (Nava et al., 2005). DFMs function to influence the host immune system via increased antibody production, up-regulation of cell-mediated immunity, promotion of epithelial barrier integrity, reduction of epithelial cell apoptosis, enhancement of dendritic cell-T cell interaction, and augmenting Toll-like receptor signaling (reviewed in Lee et al., 2010a). These activities of DFMs provide scientific basis for their use in augmenting the chicken protective immune system. Phagocytosis of fluorescent beads or GFP-labeled Salmonella Enteritidis (Higgins et al., 2007) and upregulating oxidative burst and degranulation (Farnell et al., 2006) in broiler chickens. We have recently reported that Bacillus subtilis-based DFMs stimulated different aspects of host innate and adaptive immunity i.e., humoral and cell-mediated immunity in broiler chickens (Lee et al., 2010b). Especially, serum levels of α-1-acid glycoprotein, an inflammatory marker, were reduced in DFM-fed birds, while splenic lymphocyte proliferation, intestinal intraepithelial (IEL) subpopulation, and cytokine mRNA levels in IELs were affected depending on the strains of DFMs used. We also found that dietary DFMs increased the resistance of chickens to experimental coccidiosis (Lee et al., 2007a,b, 2010c). In this study, we further examined the role of B. subtilis-based DFMs on macrophage functions, i.e., nitric oxide (NO) production and phagocytosis, two important innate immune functions of macrophages (Lillehoj and Li, 2004; Munoz-Fernandez et al., 1992; Stuehr and Marletta, 1985). Macrophage, a key component of host innate immunity, participates in host defense by secreting cytokines (e.g., IL1, IL12 and TNF) and nitric oxide (NO) that modulate inflammation and kill microbes.

2. Materials and methods

2.1. Materials

All materials were obtained from Sigma Chemical Co. (St. Louis, MO), unless otherwise indicated. Biologically active chicken
recombinant interferon gamma (IFNγ) was produced as described (Lillehoj and Choi, 1998). Briefly, IFNγ cDNA was subcloned into the pFastBac Hta vector (Life Technologies, Gaithersburg, MD), transformed into DH10Bac Escherichia coli (Life Technologies), plasmid DNA gel purified, and transfected into Spodoptera frugiperda (Sf9) cells using CellFectin (Life Technologies) according to the manufacturer’s instructions. Culture supernatant from transfected Sf9 cells served as the source of recombinant IFNγ.

2.2. Direct-fed microbials

Eight individual B. subtilis strains, designated Bs2084, LSSAO1, 3AP4, Bs18, 15AP4, 22CP1, Bs27, and Bs278, and one multiple-strain DFM product, Avicorr™ (Danisco/Agtech Products Inc., Waukesha, WI) were used. These Bacillus strains were isolated from various sources including poultry litter, swine lagoon, rumen fluid, and other agricultural environments and were selected based on their in vitro inhibitory effects on avian pathogenic E. coli or Clostridium perfringens type A (Rehberger and Jordan-Parrott, 2005). Avicorr contains equal amounts of Bs2084, LSSAO1, and 15AP4; is generally recognized as safe by the U.S. Food and Drug Administration; and is approved for feeding to animals by the Association of American Feed Control Officials.

2.3. Experimental design

Two hundred and forty day-old male broiler chicks (n = 24/group) hatched at Lonenegeek's Hatchery (Elizabethtown, PA) were weighed on arrival and randomly distributed to Petersime brooder units. At days 1 and 2 posthatch, chickens were orally administered with 5.0 × 10^8 cfu of DFM suspended in 0.5 ml of sterile distilled water to ensure the DFM intakes at early ages. The control chickens were orally gavaged with carrier only dissolved in distilled water. All birds were provided with nonmedicated mash base diets at days 1 and 2. Beginning at day 3, chickens were provided with nonmedicated mash base diets supplemented with 1.5 × 10^5 cfu/g of DFM until the end of the experiment. The control diet was formulated by mixing the base diet with carrier alone. At days 7, 14, and 21, five chickens per group were randomly chosen for blood sampling. Blood was collected from cardiac puncture with a sterile syringe containing 10 U/ml heparin immediately following euthanasia. A small portion of whole blood obtained was used to isolate plasma by gentle centrifugation and stored at −20 °C until use. The rest were pooled by treatment group and immediately used to isolate peripheral blood-derived monocytic cells (PBMC)-derived macrophages. All experimental protocols were approved by the Small Animal Care Committee of the Beltsville Agricultural Research Center.

2.4. PBMC-derived macrophage isolation

Pooled whole blood per group was diluted 1:2 with RPMI 1640 medium and peripheral blood leukocytes were collected by Ficoll–Hypaque (Sigma) density gradient centrifugation. Then, the cells were washed 3 times with calcium–magnesium-free Hank's balanced salt solution (CMF-HBSS, Sigma), and adjusted to contain 1 × 10^7 cells/ml in complete RPMI 1640. One milliliter aliquot of peripheral blood leukocytes was dispensed to a round-bottomed 24-well cell culture plate, in which some wells with glass cover slide inside were prepared for fluorescent bead-based phagocytosis assay, whereas the others for NO stimulation and phagocytosis of fluorescent bacteria. They were incubated at 41 °C in 5% CO₂ for 24 h. After incubation, non-adherent cells (e.g., lymphocytes) were removed by washing twice with RPMI-1640 containing 100 U/ml penicillin and 100 μg/ml streptomycin. Medium was replaced with complete RPMI 1640 containing no phenol red.

2.5. PBMC-derived macrophage nitric oxide production assay

Macrophages obtained at days 7, 14, and 21 were stimulated with lipopolysaccharide (LPS) of E. coli and Salmonella typhus at 5 μg/ml and 1:10 diluted chicken recombinant IFNγ for 48 h. Culture supernatants were clarified by filtering through a 0.45 μm filter and stored at −20 °C until used for NO assay.

2.6. NO assay

NO levels were measured as described (Lillehoj and Li, 2004). In brief, plasma and supernatant samples were centrifuged at 1,000g for 30 min at 4 °C, and 100 μl was mixed with an equal volume of freshly prepared Griess reagent (Sigma) containing 1% (w/v) sulfanilamide in 5% phosphoric acid and 0.1% (w/v) N-(1-naphthyl)ethylene-diamine, incubated for 10 min at room temperature, and color changes were measured at an optical density (OD) of 540 nm using a microtiter plate reader (Bio-Rad, Richmond, CA, USA). Nitrite concentrations were calculated from a standard curve using NaN3 (Green et al., 1982).

2.7. Phagocytosis assay of GFP-labeled Salmonella by PBMC macrophage

Phagocytosis of macrophages obtained at days 7 and 21 was performed as described (Raybourne et al., 2001; Okamura et al., 2005) using green fluorescence protein (GFP)-labeled Salmonella Enteritidis (SE338, phage type 4) with some modification. In brief, overnight cultures were washed twice with sterile PBS, re-suspended in PBS and adjusted to an OD 550 of 0.5. This provided an approximate count of 10^8 cfu/ml. The PBMC-derived macrophages were washed with CMF-HBSS, re-suspended at 1.0 × 10^6 cells/ml concentration in antibiotic-free RPMI-1640 with 10% FCS, and exposed to GFP-expressing SE strain 338 (Miller and Lindow, 1997) at a multiplicity of infection of 20–100. These suspensions were incubated at 37 °C and 5% CO₂ for 1 h in RPMI 1640 with 10% FBS without antibiotics. After the initial uptake, cells were washed twice with RPMI 1640 with FBS containing gentamicin (GIBCO, Grand Island, NY, USA) at 10 mg/ml and re-suspended in the same medium. These cells were analyzed for uptake of fluorescent bacteria using a FACSAria™ II flow cytometer (BD Biosciences, San Jose, CA).

2.8. Phagocytosis assay of fluorescent beads by PBMC macrophage

Phagocytosis of fluorescent beads by macrophages obtained at days 14 and 21 was assayed as described (Gilbert et al., 2008). In brief, 500 μl of yellow-green fluorescent latex beads (Sigma) were washed twice in 50 mM carbonate buffer (pH 9.0), resuspended in 5 ml of the same buffer containing 5 mg/ml of BSA and incubated at 37 °C for 2 h with constant stirring. After the incubation, they were washed 3 times with Veronal buffered sucrose saline (pH 7.3) containing 0.1% gelatin, 0.15 mM CaCl₂, and 1.0 mM MgCl₂, and resuspended in the same buffer at 1.0 × 10^6 beads/ml.

A 100 μl suspension of fluorescent beads was adjusted to contain the concentration of 2.0 × 10^8 beads/ml, sonicated and added to each well containing the adherent macrophages on glass slides in a 24-well tissue culture plate. The mixture was shaken gently, and incubated at 37 °C for 2 h. After the incubation, the mixture was washed twice with ice-cold PBS containing 0.2% EDTA and 0.1% NaN₃, fixed with methyl ethanol for 10 min, and washed 3 times. Macrophages were then counter-stained with 0.01 mg/ml of propidium iodide solution in Evan’s blue solution for 10 min, washed 3 times with ice-cold PBS, and covered with mounting medium for microscopic examination. Macrophages containing different number of 1–5, 6–10, and more than 11 fluorescent beads
3.1. Plasma NO production

Bacillus subtilis (Table 1). At day 14, NO levels were plateaued in all treatment groups compared with the control diet-fed chickens (*P<0.05) was observed in chickens fed diets containing 3AP4, 15AP4, Bs27, or Avicorr diets compared with the control group. At day 21, NO levels were increased in groups given LSSAO1, 3AP4, 15AP4, and Bs27 and Avicorr diets compared with the control group.

2.9. Statistical analysis

All values were subjected to ANOVA using SPSS 15.0 for Windows (SPSS Inc., Chicago, IL). Treatment means were tested for statistically significant differences with the multiple range test of Duncan using SPSS 15.0 for Windows (SPSS Inc., Chicago, IL). A probability of *P<0.05* was preset for statistical significance.

3. Results

3.1. Plasma NO production

At day 7, approximately 1.5-fold higher NO concentration (*P<0.05) was observed in chickens fed diets containing 3AP4, 22CP1 or Bs27 compared with the control diet-fed chickens (Table 1). At day 14, NO levels were plateaued in all treatment groups with Bs2084, 3AP4, 15AP4, and Bs27 (*P<0.05*) groups showing higher values compared with the control group. However, NO levels dropped in all groups at day 21 and remained low with a concentration of 1.6–4.4 μM.

### Table 1

<table>
<thead>
<tr>
<th>Treatments</th>
<th>NO concentration (μM)*</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.3 ± 0.2</td>
<td>7.4 ± 2.2</td>
<td>3.5 ± 0.8</td>
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<tr>
<td>Bs2084</td>
<td>6.8 ± 1.8</td>
<td>14.1 ± 1.6*</td>
<td>3.6 ± 1.6</td>
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<tr>
<td>LSSAO1</td>
<td>5.8 ± 0.8</td>
<td>7.5 ± 0.4</td>
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<td>3AP4</td>
<td>9.0 ± 1.0</td>
<td>13.1 ± 0.4</td>
<td>3.1 ± 0.7</td>
<td></td>
</tr>
<tr>
<td>Bs18</td>
<td>7.4 ± 0.5</td>
<td>9.5 ± 0.3</td>
<td>3.0 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>15AP4</td>
<td>5.3 ± 1.0</td>
<td>15.3 ± 0.9</td>
<td>2.5 ± 0.7</td>
<td></td>
</tr>
<tr>
<td>22CP1</td>
<td>7.7 ± 0.4</td>
<td>8.9 ± 2.6</td>
<td>4.4 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>Bs27</td>
<td>9.8 ± 1.7</td>
<td>11.5 ± 1.9*</td>
<td>2.6 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>Bs278</td>
<td>5.5 ± 0.4</td>
<td>10.1 ± 2.4</td>
<td>2.6 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>Avicorr</td>
<td>7.0 ± 1.6</td>
<td>6.5 ± 0.1</td>
<td>1.6 ± 0.1*</td>
<td></td>
</tr>
</tbody>
</table>

* NO levels were means (±S.D.) of 5 birds per treatment.
* Significantly increased NO concentration compared with the control group (*P<0.05*).
* Significantly decreased NO concentration compared with the control group (*P<0.05*).

was used to calculate the percentage of PBMC which engulfed different number of fluorescent beads at different times post administration of DFM (%): Percentage of macrophages engulfed fluorescent beads = (Number of macrophages engulfed certain number of fluorescent beads/total macrophages in 5 fields) × 100.

3.2. IFNγ- or LPS-induced NO production by PBMC-derived macrophages

NO production by PBMC-derived macrophages stimulated with either IFNγ or LPS-derived from E. coli or S. typhi was measured as an indicator of macrophage function. At day 7, IFNγ-induced NO production was significantly higher (*P<0.05*) in 5 (15AP4, 22CP1, Bs27, Bs278, Avicorr) of the 9 groups on DFM diets compared with the control group (Table 2). In contrast, NO production was significantly lower (*P<0.05*) in groups given Bs2084, LSSAO1, Bs18, 15AP4, Bs27, or Avicorr diets at day 14 compared with the control group. At day 21, NO levels were increased in groups given LSSAO1, Bs18, Bs27, or Avicorr diets compared with the control group.

NO production by PBMC-derived macrophages stimulated with LPS from either E. coli or S. typhi is presented in Table 2. In general, there were obvious increases (*P<0.05*) in NO production in 22CP1, Bs27, Bs278, and Avicorr groups at all time points compared with the control group. On the other hand, low NO levels in S. typhi-stimulated macrophages were seen in Bs2084, LSSAO1, and 3AP4 groups compared with the control group at day 7.

3.3. Phagocytosis of fluorescent beads by PBMC-derived macrophages

The phagocytic capacity of macrophages using fluorescent glass beads from different treatment groups is shown in Table 3. In general, percent phagocytosis by macrophages was higher in all DFM-treated groups compared with the control group. It is notable that the percentages of macrophages which engulfed more than 11 beads were higher in 6 of the DFM groups at day 14 (Bs2084, LSSAO1, 3AP4, Bs18, 15AP4, and Bs278) and day 21 (Bs2084, Bs18, 22CP1, Bs27, Bs278, and Avicorr) compared with the control group.

### Table 2

<table>
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<tr>
<th>Stimulants</th>
<th>Control</th>
<th>Bs2084</th>
<th>LSSAO1</th>
<th>3AP4</th>
<th>Bs18</th>
<th>15AP4</th>
<th>22CP1</th>
<th>Bs27</th>
<th>Bs278</th>
<th>Avicorr</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFNγ (μM)</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Day 7</td>
<td>6.3 ± 0.3</td>
<td>6.7 ± 1.4</td>
<td>6.7 ± 1.4</td>
<td>5.9 ± 0.5*</td>
<td>4.1 ± 0.2*</td>
<td>8.4 ± 0.7</td>
<td>5.3 ± 0.6*</td>
<td>6.4 ± 0.3</td>
<td>5.3 ± 1.2*</td>
<td>&lt;0.001</td>
<td></td>
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<tr>
<td>Day 14</td>
<td>5.8 ± 1.1</td>
<td>6.7 ± 0.2</td>
<td>10.0 ± 0.7</td>
<td>5.8 ± 0.1</td>
<td>9.2 ± 1.4*</td>
<td>6.3 ± 0.8</td>
<td>5.4 ± 0.2</td>
<td>7.2 ± 1.5</td>
<td>14.4 ± 0.3*</td>
<td>13.9 ± 1.5*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>E. coli LPS (μM)</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Day 7</td>
<td>6.3 ± 0.3</td>
<td>6.7 ± 1.4</td>
<td>6.7 ± 1.4</td>
<td>5.9 ± 0.5*</td>
<td>4.1 ± 0.2*</td>
<td>8.4 ± 0.7</td>
<td>5.3 ± 0.6*</td>
<td>6.4 ± 0.3</td>
<td>5.3 ± 1.2*</td>
<td>&lt;0.001</td>
<td></td>
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<tr>
<td>Day 14</td>
<td>5.8 ± 1.1</td>
<td>6.7 ± 0.2</td>
<td>10.0 ± 0.7</td>
<td>5.8 ± 0.1</td>
<td>9.2 ± 1.4*</td>
<td>6.3 ± 0.8</td>
<td>5.4 ± 0.2</td>
<td>7.2 ± 1.5</td>
<td>14.4 ± 0.3*</td>
<td>13.9 ± 1.5*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>S. typhi LPS (μM)</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Day 7</td>
<td>6.3 ± 0.3</td>
<td>6.7 ± 1.4</td>
<td>6.7 ± 1.4</td>
<td>5.9 ± 0.5*</td>
<td>4.1 ± 0.2*</td>
<td>8.4 ± 0.7</td>
<td>5.3 ± 0.6*</td>
<td>6.4 ± 0.3</td>
<td>5.3 ± 1.2*</td>
<td>&lt;0.001</td>
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<tr>
<td>Day 14</td>
<td>5.8 ± 1.1</td>
<td>6.7 ± 0.2</td>
<td>10.0 ± 0.7</td>
<td>5.8 ± 0.1</td>
<td>9.2 ± 1.4*</td>
<td>6.3 ± 0.8</td>
<td>5.4 ± 0.2</td>
<td>7.2 ± 1.5</td>
<td>14.4 ± 0.3*</td>
<td>13.9 ± 1.5*</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* The NO levels represent mean (±S.D.) of quintuplicate observations from five birds per treatment group.
* Significantly increased NO concentration compared with the control group (*P<0.05*).
* Significantly decreased NO concentration compared with the control group (*P<0.05*).

### Table 3

Effect of dietary Bacillus subtilis-based direct-fed microbials on IFNγ- and LPS-induced nitric oxide (NO) production by peripheral blood-derived monocytic cells-derived macrophage in broiler chickens.

The percentages of macrophages phagocytosed GFP-labeled Salmonella Enteritidis are shown in Fig. 1. The phagocytosis of GFP-labeled Salmonella by macrophages was markedly higher (*P<0.05*) in Bs2084, 3AP4, 15AP4, and Bs27 groups compared with the control group at day 7. At day 21, Salmonella-phagocytosed macrophages were higher in all DFM groups compared with the control group.

3.4. Phagocytosis of GFP-labeled Salmonella by PBMC-derived macrophages

The percentages of macrophages phagocytosed GFP-labeled Salmonella Enteritidis are shown in Table 3. In general, percent phagocytosis by macrophages was higher in all DFM-treated groups compared with the control group. It is notable that the percentages of macrophages which engulfed more than 11 beads were higher in 6 of the DFM groups at day 14 (Bs2084, LSSAO1, 3AP4, Bs18, 15AP4, and Bs278) and day 21 (Bs2084, Bs18, 22CP1, Bs27, Bs278, and Avicorr) compared with the control group.
DFM diets showed enhanced plasma NO levels with heightened during the first 2 weeks post hatch. For example, all groups on diseases (Kogut, 2009). The results of our study clearly demonstrated strong phagocytosis potential and engulfed significantly higher numbers of fluorescent beads and GFP-labeled Salmonella.

NO, an important mediator of innate immunity, is produced upon the stimulation of pathogen-associated molecular patterns (PAMPs) by bacterial LPS and macrophage-activating cytokines with IFNγ being the most powerful inducer. PAMPs activate nuclear factor kappaB transcription factor, NFκB, the central molecule that mediates downstream expression of a wide array of immune-related genes (Sharif et al., 2007). Among these is the inducible nitric oxide synthase (iNOS) which catalyzes the formation of NO. Indeed, it was shown that chicken primary monocytes/macrophages produced large quantities of NO when exposed to ligands of PAMPs such as Salmonella, or CpG oligodeoxynucleotides (Babu et al., 2006; He et al., 2006a,b, 2007). The primary function of NO is to kill bacteria, fungi, protozoa and tumor cells via the production of the potent oxidant peroxynitrite following radical–radical reaction with superoxide (Lillehoj and Li, 2004; Rubbo et al., 1994; Tizard, 2007). In this study, significant amount of NO was produced in IFNγ- and LPS-stimulated PBMC-derived macrophages from broiler chickens fed B. subtilis-based DFM diets. In concordance with these results, increased NO production by Bifidobacterium and Lactobacillus DFM-stimulated macrophage has been noted elsewhere (Kim et al., 2007; Korhonen et al., 2001).

Fig. 1. Effect of dietary B. subtilis-based direct-fed microbials on phagocytosis of GFP-labeled Salmonella Enteritidis by peripheral blood-derived mononucelic cells-derived macrophage in broiler chickens. Each bar represents the mean ± S.D. (n=5). The asterisk (*) denotes significantly increased phagocytosis (%) compared with the control group (P<0.05).

Table 3

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Percentage of phagocytosis (%) a</th>
<th>Day 14</th>
<th>Day 21</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1–5 Beads</td>
<td>6–10 Beads</td>
<td>11 and more beads</td>
</tr>
<tr>
<td>Control</td>
<td>29.8 ± 3.4</td>
<td>7.1 ± 1.3</td>
<td>7.2 ± 0.9</td>
</tr>
<tr>
<td>BS2084</td>
<td>36.0 ± 4.3</td>
<td>15.4 ± 1.2</td>
<td>27.7 ± 3.1</td>
</tr>
<tr>
<td>LSSA01</td>
<td>27.5 ± 2.4</td>
<td>15.6 ± 2.1</td>
<td>38.6 ± 2.2</td>
</tr>
<tr>
<td>3AP4</td>
<td>40.0 ± 3.5</td>
<td>10.4 ± 1.0</td>
<td>16.0 ± 0.2</td>
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<tr>
<td>BS18</td>
<td>28.1 ± 1.3</td>
<td>13.8 ± 4.3</td>
<td>29.7 ± 0.9</td>
</tr>
<tr>
<td>15AP4</td>
<td>33.4 ± 1.9</td>
<td>13.9 ± 1.7</td>
<td>28.2 ± 1.4</td>
</tr>
<tr>
<td>22CP1</td>
<td>39.4 ± 4.6</td>
<td>13.1 ± 4.5</td>
<td>25.5 ± 2.6</td>
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<td>BS27</td>
<td>32.6 ± 2.5</td>
<td>10.9 ± 2.5</td>
<td>11.6 ± 2.0</td>
</tr>
<tr>
<td>BS278</td>
<td>35.1 ± 1.1</td>
<td>17.6 ± 0.9</td>
<td>26.5 ± 5.6</td>
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<td>Aovicorr</td>
<td>35.1 ± 3.7</td>
<td>7.7 ± 1.5</td>
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</tr>
</tbody>
</table>

* The percentages of phagocytosis represents mean (±S.D.) of quintuplicate observations from five birds per treatment group.
* Significantly increased phagocytosis (%) compared with the control group (P<0.05).

4. Discussion

This study was undertaken to investigate whether dietary B. subtilis-based DFMs would augment macrophage function of broiler chickens. Macrophages and heterophils, the latter being the avian equivalent to mammalian neutrophils play an important role in innate immune response in poultry and are capable of producing many biologically active effector molecules many of which can greatly influence the local and systemic immune responses (Qureshi, 2003). Furthermore, enhanced heterophil oxidative burst and degranulation was observed in broiler chickens fed B. subtilis-, Lactococcus lactis- or L. acidophilus-supplemented diets (Nunes et al., 2006). On the other hand, Nunes et al. (2009) failed to observe the influence of dietary DFMs on the phagocytosis of macrophages.
macrophages in broiler chicks. Possible reasons for this discrepancy may include host immune status, bacterial strains, age of birds, and other environmental factors. Indeed, our study indicated that the observed immunomodulatory effect of *B. subtilis*-based DFMs on macrophage function is dependent on the strain of *B. subtilis* used.

Taken together, this study documented the immunomodulatory effect of *B. subtilis*-based DFMs on macrophages by their NO production and enhanced phagocytic activity. These new scientific findings provide an environmentally safe and rational basis to use dietary DFMs to enhance host innate immunity and to lessen the use of antibiotics in animal production. In view of increasing scientific evidence elucidating the role of the gut microbiota on host immunity (Sekirov and Finlay, 2009), further studies to identify properties and biological effects of different bacterial strains on gut physiology and immune system development should be encouraged.

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References


