Effect of *Bacillus*-based direct-fed microbials on *Eimeria maxima* infection in broiler chickens

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INTRODUCTION

➢ **Enteric diseases**
- Commercial poultry production practices cause significant losses in productivity, increased mortality, and contamination of food products for human consumption.
- *Eimeria* spp. are etiologic agents of avian coccidiosis, an intestinal disease with an estimated annual loss of more than $3 billion worldwide.
- Pre-exposure to certain species of *Eimeria* has been implicated in promoting necrotic enteritis (NE) and gangrenous dermatitis (GD).
- *Clostridium*-related pathogens such as GD, coccidiosis, NE, enteritis of unknown etiologies, and collibacillosis in broiler chickens.

➢ **Direct-fed microbials (DFMs)**
- Probiotics have been successfully used to improve the balance of gut microflora.
- Spores of *Bacillus cereus*, *B. subtilis*, and *B. clausii* have been used as DFMs for food animals and humans and are known to enhance protective immune response.
- Dietary supplementation with a *Bacillus*-based DFM was shown to improve feed conversion in poultry and to beneficially alter the gastrointestinal microflora to reduce colonization by avian pathogenic *Escherichia coli* and *Clostridium perfringens* type A.
- *Bacillus subtilis*-based DFMs stimulated different aspects of host humoral and cell-mediate immunity in broiler chickens.

➢ Since DFMs can augment the host immunity, we hypothesized that *Bacillus subtilis*-based DFMs as an immunomodulating agent would increase the resistance of chickens to experimental coccidiosis.

OBJECTIVE

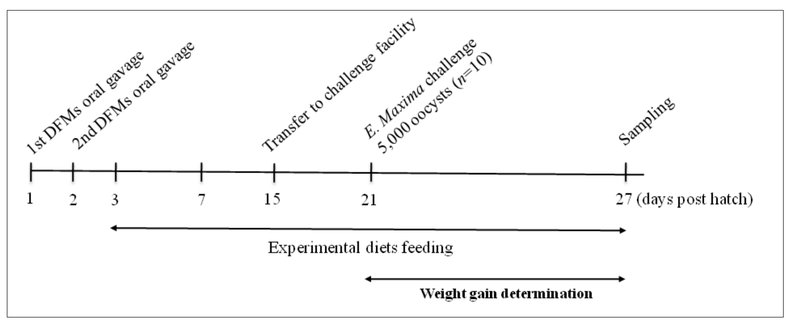
➢ To test whether *Bacillus*-based DFMs would increase the resistance of chickens to coccidiosis.

MATERIALS AND METHODS

1. Direct-fed microbials

➢ Eight individual *Bacillus subtilis* strains (Bs2084, LSSAO1, 3AP4, Bs18, 15AP4, 22CP1, Bs27, Bs278), and one multiple-strain DFM product, AVICORR (Danisco/Agtech, Waukesha, WI) were used.
➢ The individual *Bacillus* strains were isolated from various agricultural environments that included poultry litter, a swine lagoon, and rumen fluid.
➢ AVICORR® is a commercially available multi-strain DFM product and produced by the Danisco/Agtech company (Waukesha, WI).
➢ Certain strains of *B. subtilis* have been selected as candidate DFMs on the basis of their *in vitro* inhibitory effect on avian pathogenic bacteria. Also dietary supplementation with a *Bacillus*-based DFM has shown to improve feed conversion in poultry and to beneficially alter the gastrointestinal microflora to reduce colonization by avian pathogenic *E. coli* and *Clostridium perfringens* type A

	Cont	EM Cont	DFM treatments								
DFM	-	-	Bs-2084	LSS-AO1	3AP4	Bs18	15AP4	22CP1	Bs27	Bs278	Avi-corr
E. Maxima	-	+	+	+	+	+	+	+	+	+	+



2. Birds, diets, and experimental design

➢ Used one-day-old broiler chickens (n = 250) from Longenecker's Hatchery (Elizabethtown, PA)
➢ At days 1 and 2 post-hatch
- 5.0 × 10^6 colony forming units (CFU) of DFMs was suspended in 0.5 ml of sterile distilled water and administered to chicks (n = 24/group) by oral gavage
- Controls (n = 34) given only carrier dissolved in water
➢ Beginning at day 3 post-hatch
- Chicks were fed *ad libitum* a non-medicated mash base diet supplemented with 1.5 × 10^5 CFU/g of DFMs until the end of the experiment
- Control diet was formulated by mixing the base diet with carrier alone
➢ At day 21 post-hatch
- 10 birds were randomly selected from each dietary treatment and orally infected with 5.0 × 10^3 sporulated oocysts of *E. maxima* (EM).
- Ten naïve birds fed a non-supplemented diet were also randomly selected.
➢ Body weight was measured on days 21 and 27 (0 and 6 dpi).
➢ At day 27 post-hatch
- Blood, spleens, and small intestines (n = 5) were collected.
- All experimental protocols were approved by the Small Animal Care Committee of Beltsville Agricultural Research Center.

3. Lesion scores

➢ A 20-cm portion of the intestine that was anterior to and posterior to the Meckel's diverticulum was removed cut longitudinally.
➢ Intestinal contents were gently removed and lesion scores from 0 to 4 in ascending order of severity as described by Johnson and Reid were independently made by 3 observers in a blinded fashion with no knowledge of treatment group.

4. Serum nitric oxide (NO) levels

➢ Serum (100 µl) was mixed with an equal volume of freshly prepared Griess reagent (Sigma, St. Louis, MO), and incubated for 10 min at room temperature. The optical density at 540 nm was determined with an automated microtiter plate reader (Bio-Rad, Hercules, CA).
➢ Nitrite concentrations were calculated from a standard curve generated with $NaNO_2$.

5. Serum anticoccidial antibody levels

➢ ELISA was used to measure antibody levels against the *Eimeria* recombinant profilin protein (3-1E) in sera collected at 6 days PI.
➢ Microtiter plates were coated overnight with 10 µg/well of purified recombinant 3-1E protein. The plates were washed with PBS containing 0.05% Tween 20 (PBS-T) and blocked with PBS containing 1% BSA.
➢ Sera (100 µl/well) were incubated for 2 h at room temperature with gentle agitation.
➢ The plates were washed with PBS-T, and bound antibody was detected with peroxidase-conjugated rabbit anti-chicken IgG (Sigma) and peroxidase-specific substrate. OD_{450} values were measured with a microplate reader.

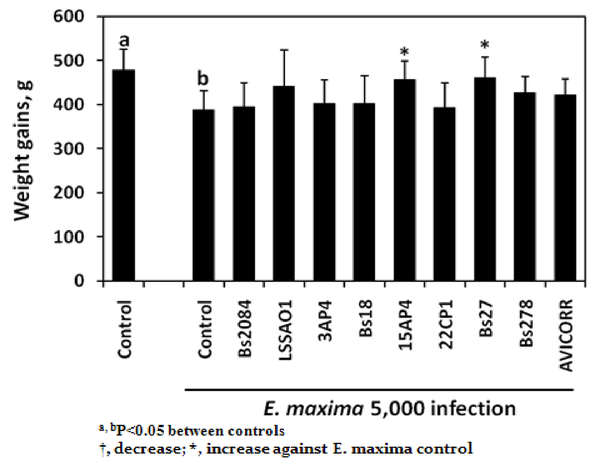
6. Concanavalin A- and 3-1E-induced spleen lymphocyte proliferation

➢ Splenocytes obtained from single cell suspensions, seeded in a 5 × 10^5/well in 96-well microtitre plates and incubated with medium alone (control), concanavalin A (ConA, 2.5 µg/ml), or recombinant 3-1E protein (10 µg/ml) in a humidified incubator at 41 °C with 5% CO_2 for 48 h.
➢ Cell proliferation at 48 h was expressed as the stimulation index (SI), calculated as the ratio of the mean OD_{450} value of mitogen-stimulated cells divided by the mean OD_{450} value of medium alone-stimulated cells.

7. Statistical analysis

➢ All data were subjected to one-way analysis of variance using SPSS 15.0 for Windows (SPSS Inc., Chicago, IL).
➢ Mean values of treatment groups were compared using the Duncan's multiple range test and differences were considered statistically significant at $P < 0.05$.

RESULTS



a, bP<0.05 between controls
†, decrease; *, increase against E. maxima control

Fig. 1. Body weight gain between 0 and 6 dpi

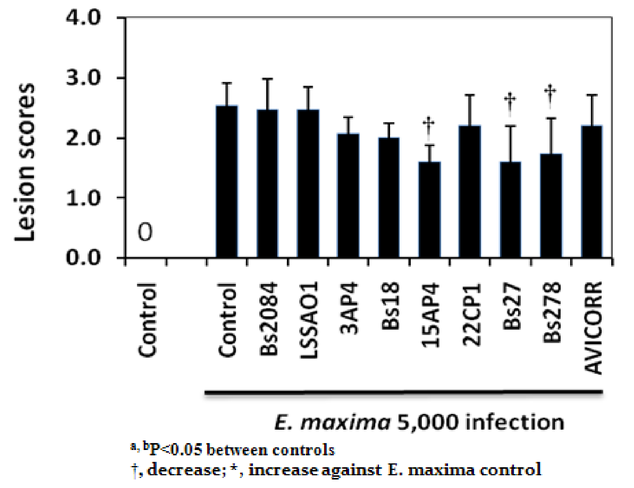


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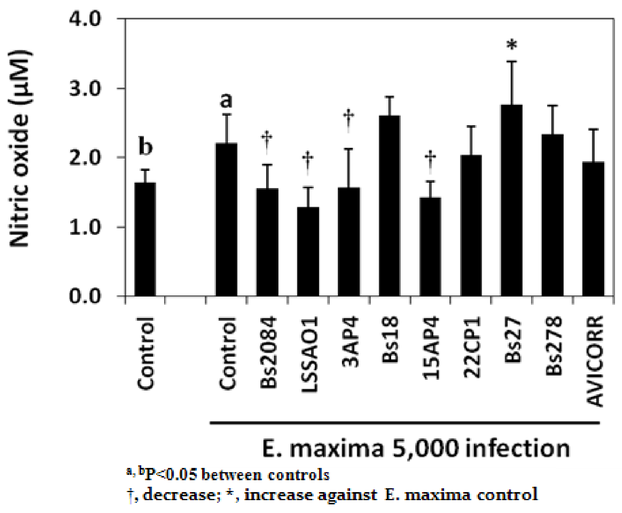
Fig.2. Lesion scores at 6 dpi



a, bP<0.05 between controls
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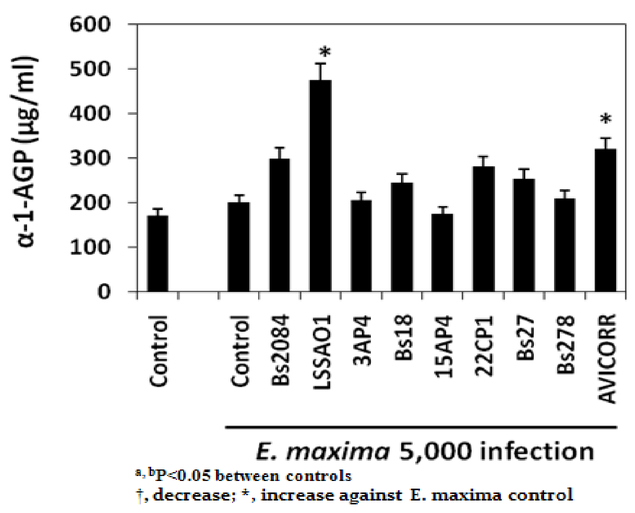
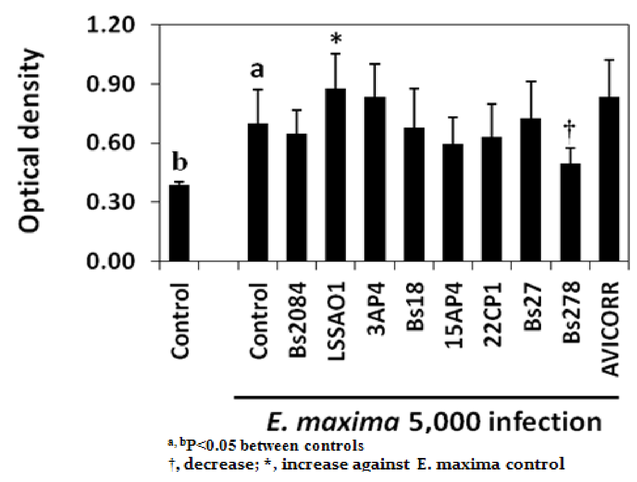
Fig. 3. Serum nitric oxide level at 6dpi



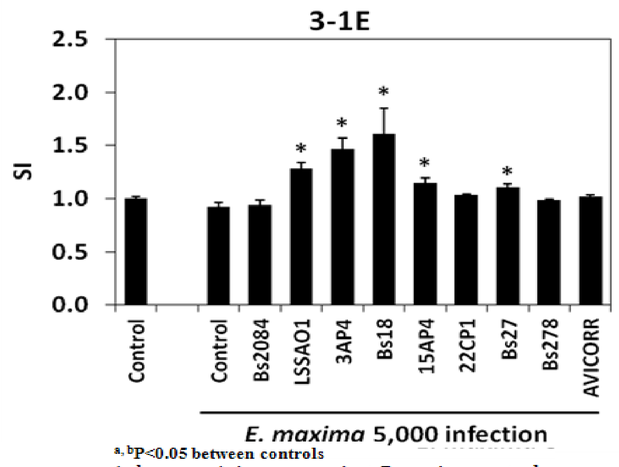
a, bP<0.05 between controls
†, decrease; *, increase against E. maxima control

Fig.4. Serum acute phase protein level at 6 dpi



a, bP<0.05 between controls
†, decrease; *, increase against E. maxima control

Fig. 5. Serum antibody response against 3-1E (profilin) at 6 dpi.



a, bP<0.05 between controls
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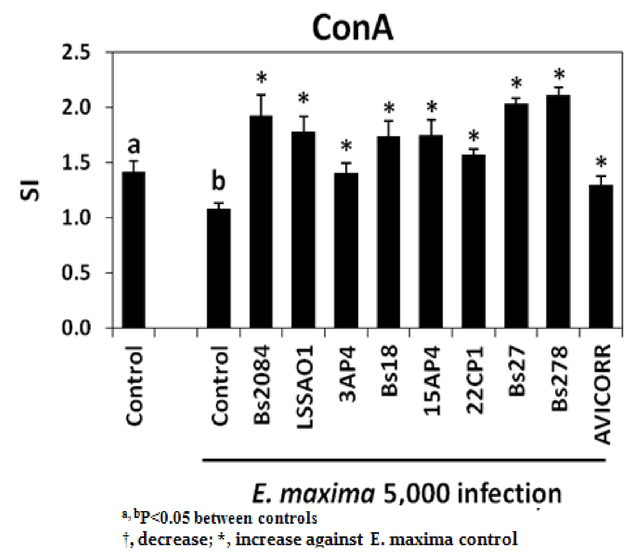
Fig. 6. Antigen-specific splenocyte proliferation at 6 dpi .



a, bP<0.05 between controls
†, decrease; *, increase against E. maxima control

Fig.7. T-cell mitogenic splenocyte proliferation

Table 1. Summary of experiment

Measure	Bs2084	LSSAO1	3AP4	Bs18	15AP4	22CP1	Bs27	Bs278	AVICORR
BW		↑			↑↑		↑↑	↑	↑
Lesion			↓	↓	↓↓		↓↓	↓↓	↓
NO	↓↓	↓↓	↓↓	↑	↓↓		↑↑		↓
α-1-AGP	↑	↑↑				↑			↑↑
Anti-body		↑↑	↑					↓↓	↑
SI/ 3-1E		↑↑	↑↑	↑↑	↑↑	↑	↑↑		↑
SI/ ConA	↑↑	↑↑	↑↑	↑↑	↑↑	↑↑	↑↑	↑↑	↑↑

↑: numerical increase; ↑↑: significant increase vs. the *E. maxima* control , ↓: numerical decrease; ↓↓: significant decrease vs. the *E. maxima* control

CONCLUSION

Dietary *Bacillus*-based DFMs
1. **Reduced the clinical signs of *E. maxima* infection**
2. **Enhanced host innate and acquired immunity**
3. **Effects were bacterial strain-dependent**

ACKNOWELDMENTS

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