Evaluation of *Bacillus* species as potential candidates for direct-fed microbials in commercial poultry

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ABSTRACT Increasing sociopolitical concerns with antibiotic use have led to investigations of potential alternatives for food safety and growth promotion. Direct-fed microbials (DFM) including spore-based probiotics are amenable to feed inclusion and are extremely stable. We isolated several *Bacillus* spp. from environmental and poultry sources and tested them for their ability to reduce *Salmonella* in vitro. In a preliminary in vivo trial, day-of-hatch chicks and poults were randomly assigned to the following treatments (24 birds/treatment): control and one of 8 DFM candidates at 10^6 spores/g of feed. Chicks and poults were tagged, weighed, and orally challenged with *Salmonella* Typhimurium (ST). Body weight gain and ST recovery were measured 11 d posthatch. Total percentages of ST-positive crop and ceca were significantly lower ($P < 0.05$) in at least 3 DFM candidates compared with control. Additionally, beneficial effects on BW gain were observed in at least 5 DFM candidates ($P < 0.05$) compared with control. In a second study, birds treated with NP122 (identified as *Bacillus subtilis*) had significantly lower ($P < 0.05$) cecal ST than control and benefitted BW gain irrespective of the presence or absence of a *Salmonella* challenge. In conclusion, NP122 markedly reduced ST recovery and increased BW gain in both chicks and poults. This provides preliminary evidence that this isolate may have potential use as a DFM in poultry.

Key words: *Bacillus*, spore, probiotic, *Salmonella*, poultry

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INTRODUCTION

The use of antibiotics as prophylactic and growth-promoting compounds has long been practiced in commercial poultry farming. However, increased antibiotic use has led to the development of antibiotic-resistant microorganisms, which has led to failure of antibiotic treatments, production losses, and the risk of zoonotic infections (Cartman et al., 2008). In addition, intense rearing practices have led to an increase in stress, which may further lead to decreased immune function and enable colonization by pathogens. This may pose a severe health hazard for birds and consumers of poultry products (O'Dea et al., 2006). Therefore, an urgent need exists to find alternative strategies that can effectively control pathogens and retain growth-promoting properties similar to antibiotics. In the poultry industry, it is essential that the method of application be simple, preferably in the form of feed additives (DuPont, 2007).

With regards to this, the use of direct-fed microbials (**DFM**) has earned attention as a viable alternative to traditional antibiotic therapies. Direct-fed microbials comprise a variety of beneficial bacteria known to have positive effects on health and performance when administered in appropriate quantities. Some commonly used DFM strains of bacteria include multiple strains of *Lactobacillus*, *Pediococcus*, *Bifidobacterium*, and *Bacillus* spp. that have been successful in animals and humans (Zani et al., 1998; Ouwehand et al., 2002; O'Dea et al., 2006). *Bacillus* are aerobic, endospore-forming bacteria that are well defined and have recently shown tremendous promise as DFM because of their inherent capacity to form spores that can withstand harsh environmental stress and transitions during storage and handling (Cartman et al., 2008). This makes the use of *Bacillus*-based DFM amenable to commercial operations as feed additives.

A large number of *Bacillus* spp. are present as saprophytes in soil and are known to have existed 250 million years ago (Vreeland et al., 2000). *Bacillus* spores are extremely suitable DFM candidates because of their longevity and stability. *Bacillus* spp. can be found in the normal intestinal flora of poultry and are capable of germinating and resporulating in the gastrointestinal

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tract of chickens (Hoa et al., 2000; Vreeland et al., 2000; Barbosa et al., 2005; Tam et al., 2006; Cartman et al., 2008). Spores are known to withstand the process of pelletizing feed and, once ingested, germinate in the gastrointestinal tract because of the influence of pH, nutrients, and other relevant factors. In their vegetative form, *Bacillus* spp. produce extracellular enzymes that may enhance digestibility and absorption of nutrients in addition to overall immune function of the gut (Samanya and Yamauchi, 2002; Chen et al., 2009).

Competitive exclusion of pathogens is a popular hypothesis to explain the action of probiotics (Patterson and Burkholder, 2003; Leser et al., 2008). Even though the process has been well demonstrated in *Lactobacillus* spp., some evidence exists that *Bacillus* spp. may have the same mode of action (Barbosa et al., 2005). Despite the plethora of beneficial effects of DFM observed, in vivo mechanisms of action have not been clearly elucidated and this may be a significant area of future research (Corr et al., 2007).

One of the most common bacterial pathogens in poultry is *Salmonella*. In addition to causing losses to the poultry industry through decreases in performance (Callaway et al., 2008), contamination of poultry products by *Salmonella* poses a severe health hazard to humans (Kimura et al., 2004). Foodborne *Salmonella* is the most common cause of human salmonellosis. It is well documented that poultry and poultry products continue to be major contributors, with raw or undercooked eggs and poultry meat being predominant contaminants (Revolledo et al., 2009; Cox and Pavic, 2010). The ceca are preliminary sites of *Salmonella* colonization, although other organs are also associated with the presence of *Salmonella* (Byrd et al., 2002). Furthermore, stress and feed withdrawal are commonly associated with *Salmonella* colonization of birds at processing age (Ramirez et al., 1997; Ricke, 2003; Burkholder et al., 2008).

Increased use of antimicrobials to control *Salmonella* populations in poultry has led to the development of resistance and the risk of zoonotic infections in humans (Antunes et al., 2003). The application of DFM may be a viable alternative to antibiotics to control enteric pathogens such as *Salmonella*. In our studies, several *Bacillus* spp. were isolated from poultry and environmental sources and tested for their ability to primarily inhibit *Salmonella* colonization in birds. Additionally, beneficial effects on BW gain were evaluated in broilers and turkey poults.

MATERIALS AND METHODS

Isolation and Characterization of Bacillus spp.

Previous research done in this laboratory focused on isolation of several *Bacillus* spp. from environmental and poultry sources (Wolfenden et al., 2010). Identification was carried out using a bioMérieux API 50 CHB test kit (catalog no. 50430, bioMérieux, Marcy l'Etoile, France) to confirm generally recognized as safe status. For our preliminary experiment, 8 isolates (Table 1) were chosen based on consistent in vitro activity against *Salmonella* spp., *Clostridium* spp., and *Campylobacter* spp. as described by Wolfenden et al. (2010) (data not shown). Additionally, the ability of these candidates to grow to high numbers (approximately 10^9 to 10^{11} spores/g) in a solid-state fermentation medium made them suitable to be evaluated in vivo (Wolfenden et al., 2010).

Table 1. Identification of candidate *Bacillus* spp. by bioMérieux API 50 CHB[1]

Bacillus designation	API identification	Identification (%)
NP119B	*Bacillus subtilis*	82.8
NP117B	*Bacillus subtilis*	98.4
NP124	*Bacillus subtilis*	94.1
NP121	*Paenibacillus polymyxa*	58.1
MM01-09	*Bacillus subtilis*	98.2
MM65	*Brevibacillus laterosporus*	99.9
NP122	*Bacillus subtilis*	98.2
JH33	*Bacillus subtilis*	99.1

[1]Identification was carried out using a bioMérieux API 50 CHB test kit (catalog no. 50430, bioMérieux, Marcy l'Etoile, France).

Feed Preparation and Spore Treatment

All candidate *Bacillus* spp. were mixed in the feed at a final concentration of 10^6 spores/g of finished feed according to previously described methods (Wolfenden et al., 2010). Briefly, candidate DFM were individually grown in a solid-state fermentation medium comprising rice straw, wheat bran, and liquid media at 40% by weight. After incubation at 37°C for 24 h followed by 30°C for another 72 h, the cultures were dried at 60°C. Following this, the cultures were aseptically ground into a fine powder to generate stable spores (approximately 10^9 to 10^{11} spores/g). Freshly prepared, unmedicated corn–soy-based starter feed that met or exceeded NRC (1994) requirements was used for all bird trials. Spores were mixed into the feed using a rotary mixer for 15 min. The mixing process was carried out 1 d before the bird trial and feed samples were titrated to confirm the desired concentration of spores (10^6 spores/g). Briefly, for each DFM candidate, samples of feed containing the respective DFM culture were taken and a 1:10 dilution was made with saline. All samples were subject to 100°C for 10 min, enumerating using 10-fold dilutions, and plate counting following overnight incubation at 37°C on tryptic soy agar plates (data not shown).

Experimental Birds

Day-of-hatch Cobb male chicks (Cobb-Vantress Inc., Siloam Springs, AR) and Hybrid male poults (Cargill

Turkey Division LLC, Gentry, AR) obtained from a local hatchery were used for all bird experiments. Chicks and poults were confirmed negative for *Salmonella* on arrival. Briefly, 10 chicks and 10 poults were randomly chosen and killed and their ceca and cecal tonsils were aseptically removed and enriched in 10 mL of tetrathionate broth (catalog no. 210420, Becton Dickinson, Sparks, MD) and incubated at 37°C for 24 h. Following this, enrichment samples were streaked on brilliant green agar (**BGA**; catalog no. 228530, Becton Dickinson) plates containing 25 μg/mL of Novobiocin (**NO**; catalog no. N1628, Sigma, St. Louis, MO). After incubation at 37°C for 24 h, the plates were observed for *Salmonella* presence or absence. All bird handling procedures were in compliance with the Institutional Animal Care and Use Committee at the University of Arkansas (Fayetteville).

Challenge Organism

A primary poultry isolate of *Salmonella* Typhimurium (**ST**) selected for resistance to nalidixic acid (**NA**) served as the challenge organism. The amplification protocol for preparation of challenge inoculum has been described in detail (Bielke et al., 2003). Briefly, an aliquot of ST was thawed and 100 μL of culture was inoculated into 10 mL of tryptic soy broth (catalog no. 211822, Becton Dickinson) and incubated at 37°C for 24 h. This was followed by 3 passages at intervals of 8 h into fresh tryptic soy broth. Following the last pass, cells were washed with sterile saline (3×) by centrifugation (1,864 × *g*, 4°C, and 15 min). The approximate concentration of ST was estimated spectrophotometrically (Spectronic 20D; Thermo Scientific, Waltham, MA) at 625 nm and adjusted to a previously determined standard curve. In addition, the ST stock solution was serially diluted and plated on BGA with 25 μg/mL of NO and 20 μg/mL of NA (catalog no. N4382, Sigma) to determine actual concentration.

Experiment 1

For the preliminary trial, day-of-hatch chicks (n = 108) and poults (n = 108) were randomly selected, neck tagged, weighed, and orally challenged with a 0.25-mL solution of ST to obtain a dose of 1.2×10^5 cfu/bird. Twelve chicks and 12 poults were placed into each of the appropriate pens (0.84 m^2) by treatment on fresh pine litter. Chickens and poults were placed together in the same pen and pens were separated by treatment. Feed and water were provided ad libitum and treatment groups were as follows: control (basal diet, no spores), NP119B, NP117B, NP124, NP121, MM01-09, MM65, NP122, and JH33 (see Table 1 for treatment descriptions). Candidate DFM were fed for the entire study period. Body weight was recorded 11 d posthatch and total BW gain was determined. All birds were killed by CO_2 asphyxiation; whole crops and ceca (n = 10/treatment) were aseptically removed for *Salmonella* recovery. Briefly, crop samples were placed in 10 mL of tetrathionate broth for enrichment and incubated at 37°C for 24 h. Ceca were homogenized and diluted with saline (1:4 by weight) and 10-fold dilutions were plated on BGA with NO and NA and incubated at 37°C for 24 h to enumerate total *Salmonella* colony-forming units. Cecal samples were enriched in double-strength tetrathionate broth and incubated at 37°C for 24 h. Following this, crop and ceca enrichment samples were streaked on BGA NO and NA plates and incubated at 37°C for 24 h to confirm presence or absence of typical lactose-negative colonies of *Salmonella*.

Experiment 2

In a second trial, chicks (n = 320) and poults (n = 320) were randomly selected, neck tagged, weighed, and placed into one of the following 4 treatments: control (no spores), NP122 only, ST only, and ST + NP122 (20 chicks and 20 poults/replicate × 4 replicates/treatment). Birds that received ST were orally challenged with a 0.25-mL solution containing 1.2×10^5 cfu of viable bacteria whereas control and NP122-only groups were sham challenged with saline on day of hatch. The birds were separated by treatment and placed in pens (1.95 m^2; n = 40/pen) on fresh pine litter with unrestricted access to feed and water. Candidate DFM were fed from d 1 through the entire study period. At 11 d posthatch, all birds were weighed and 5 chicks and 5 poults from each replicate pen were randomly chosen and killed by CO_2 asphyxiation. Enumeration and *Salmonella* recovery in crop and ceca were performed in the same manner as that of experiment 1. At 21 d posthatch, all birds were weighed to determine total BW gain and were killed.

Statistical Analysis

Body weight gain from these studies was subject to ANOVA using initial BW as a covariate. Cecal colony-forming unit data were converted to $\log_{10}$ colony-forming unit numbers. All data were then compared using the GLM procedure of SAS 9.2 (SAS Institute, Cary, NC) with significance reported at $P < 0.05$. The percentage recovery of *Salmonella* was compared using the chi-squared test of independence testing all possible group combinations to determine significance ($P < 0.05$) for these studies (Zar, 1984).

RESULTS

Experiment 1

Body weight was recorded at placement and at 11 d posthatch to determine total BW gain. Two of the 8 DFM candidates in chicks and 5 DFM candidates in poults had significantly higher ($P < 0.05$) BW gain 11

Table 2. Effect of direct-fed microbial candidates on BW and BW gain in broiler chicks or turkey poults from placement (d 0) to termination (d 11) in experiment 1[1]

	Chicks		Poults	
Treatment[2]	BW d 0 (g)	BW gain d 11 (g)	BW d 0 (g)	BW gain d 11 (g)
Control	47.5 ± 1.3	151.6 ± 10.5^{c}	50.3 ± 1.7	140.4 ± 6.3^{cd}
NP119B	44.9 ± 1.2	163.5 ± 11.8^{abc}	53.7 ± 1.2	163.6 ± 10.7^{ab}
NP117B	44.8 ± 1.2	162.4 ± 13.1^{abc}	51.0 ± 0.8	167.6 ± 7.9^{ab}
NP124	46.5 ± 1.4	186.4 ± 11.8^{ab}	53.9 ± 1.2	149.1 ± 9.0^{bc}
NP121	45.5 ± 0.6	139.0 ± 11.8^{c}	53.8 ± 1.3	128.7 ± 6.3^{d}
MM01-09	45.8 ± 0.6	147.1 ± 10.6^{c}	51.4 ± 1.1	151.5 ± 6.5^{abc}
MM65	45.6 ± 1.1	159.7 ± 14.9^{bc}	52.8 ± 1.2	164.6 ± 5.7^{ab}
NP122	45.3 ± 0.7	193.1 ± 12.0^{a}	53.3 ± 0.9	171.3 ± 5.7^{a}
JH33	47.4 ± 1.0	163.4 ± 11.6^{abc}	53.8 ± 0.9	170.7 ± 4.7^{a}

$^{a–d}$Means within a column with different superscripts differ ($P < 0.05$).

[1]Values are expressed as mean ± SE (n = 12 birds/treatment). All poults were challenged with 1.2×10^5 cfu/poult of *Salmonella* Typhimurium at placement. Candidate *Bacillus* spores were mixed in the feed at 10^6 spores/g.

[2]See Table 1 for treatment descriptions.

d posthatch compared with control. Total BW gain in NP122 chicks and poults was significantly higher ($P < 0.05$) compared with control whereas some of the other candidates enhanced performance in either chicks or poults but not both (Table 2).

Crop and cecal samples were enriched overnight in broth and streaked on BGA NO–NA plates to confirm presence or absence of ST. Recovery of ST was significantly lower ($P < 0.05$) in the crop of chicks fed 5 of the 8 DFM candidates (Table 3) and MM65 and NP122 in the crop of poults compared with control (Table 3). No significant differences ($P > 0.05$) were found in terms of reduction in cecal incidence of *Salmonella* in chicks, whereas candidate NP122 significantly reduced ($P < 0.05$) cecal *Salmonella* in poults (Table 3). Enumeration of *Salmonella* colony-forming units in ceca of chicks and poults showed no significant differences compared with control ($P > 0.05$; Table 4).

Experiment 2

The previously observed beneficial effects of NP122 on reduction of *Salmonella* and benefits on BW gain led to the evaluation of NP122 in the second study. Body weights were recorded at placement and 11 and 21 d posthatch. Body weight data at placement were analyzed as a covariate and were not significantly different in either chicks ($P = 1.83$) or poults ($P = 0.7$). In chicks, BW or BW gain was not different between the 2 ST challenged groups treated with or without NP122 at any point of time in the study. Poults challenged with ST and fed NP122 had significantly higher ($P < 0.05$) BW gain at 11 d posthatch compared with poults challenged with ST only. However, at 21 d posthatch, no significant differences ($P > 0.05$) were found in BW gain between the 2 ST challenged groups (Table 5).

Total cecal *Salmonella* colony-forming units at 11 d posthatch was significantly lower ($P < 0.05$) in both chicks and poults fed NP122, with an approximately 1.5 log reduction compared with the ST-only chicks and poults (Table 6). *Salmonella* incidence was significantly reduced ($P < 0.05$) in the crop of chicks fed NP122 compared with the ST-only group at 11 d posthatch, whereas no significant differences ($P > 0.05$) were found in the crop of poults or ceca of chicks and poults in terms of *Salmonella* incidence (Table 7). Non-

Table 3. Effect of direct-fed microbial candidates on recovery of *Salmonella* Typhimurium in broiler chicks and turkey poults at 11 d posthatch in experiment 1[1]

	Chicks		Poults	
Treatment[2]	Crop	Ceca	Crop	Ceca
Control	70	55	65	85
NP119B	10*	50	70	80
NP117B	40	60	50	60
NP124	70	90	70	90
NP121	80	60	60	90
MM01-09	10*	70	30	90
MM65	0*	20	10*	50
NP122	10*	50	10*	20*
JH33	30*	100	30	70

[1]Data are expressed as total percentage positive for *Salmonella* (n = 10/treatment).

[2]See Table 1 for treatment descriptions.

*Significantly different from control ($P < 0.05$).

Table 4. Effect of direct-fed microbial candidates on *Salmonella* Typhimurium cecal colony-forming units in broiler chicks and turkey poults at 11 d posthatch in experiment 1[1]

Treatment[2]	Chicks	Poults
Control	2.3 ± 0.4^{abc}	2.6 ± 0.3^{ab}
NP119B	1.3 ± 0.5^{c}	2.6 ± 0.5^{ab}
NP117B	2.1 ± 0.5^{abc}	2.9 ± 0.5^{ab}
NP124	3.4 ± 0.4^{a}	3.6 ± 0.3^{a}
NP121	2.0 ± 0.6^{abc}	2.6 ± 0.3^{ab}
MM01-09	2.6 ± 0.7^{abc}	2.5 ± 0.3^{ab}
MM65	1.8 ± 0.5^{bc}	1.7 ± 0.5^{b}
NP122	1.7 ± 0.6^{bc}	2.6 ± 0.6^{ab}
JH33	3.2 ± 0.3^{a}	2.1 ± 0.6^{b}

$^{a–c}$Means within a column with different superscripts differ significantly ($P < 0.05$).

[1]Data are expressed as $\log_{10}$ colony-forming unit counts of *Salmonella* in ceca samples (n = 10/treatment).

[2]See Table 1 for treatment descriptions.

Table 5. Effect of NP122 on BW and BW gain in broiler chicks and turkey poults from placement (d 0) to termination (d 21) in experiment 2[1]

Treatment[2]	BW d 0 (g)	BW gain d 11 (g)	BW gain d 21 (g)
Chicks			
Negative control	37.9 ± 0.2[a]	128.1 ± 3.9[b]	408.0 ± 13.5[b]
NP122 only	38.1 ± 0.2[a]	148.0 ± 3.1[a]	433.5 ± 10.4[ab]
ST only	38.1 ± 0.2[a]	142.3 ± 4.1[a]	428.4 ± 11.0[ab]
ST + NP122	36.3 ± 0.2[b]	151.6 ± 3.9[a]	439.2 ± 6.6[a]
Poults			
Negative control	56.0 ± 0.5[b]	157.5 ± 4.2[ab]	356.1 ± 5.5[ab]
NP122 only	54.9 ± 0.5[b]	162.6 ± 3.4[ab]	372.3 ± 6.3[a]
ST only	56.9 ± 0.5[a]	152.5 ± 4.2[b]	345.0 ± 6.2[b]
ST + NP122	55.8 ± 0.4[b]	165.6 ± 3.3[a]	346.2 ± 5.7[b]

[a,b]Means within a column with different superscripts differ significantly ($P < 0.05$; n = 160/treatment on d 11 and 120/treatment on d 21).

[1]Values are expressed as mean ± SE. Challenged birds received 1.2×10^5 cfu of *Salmonella* Typhimurium (ST) orally at placement. NP122 was mixed in the feed at 10^6 spores/g.

[2]See Table 1 for NP122 description.

challenged controls were tested and were negative for ST (data not shown).

DISCUSSION

The use of antibiotics to control pathogens and promote growth in livestock has been practiced for more than 5 decades. However, increasing sociopolitical concerns regarding antibiotic use has prompted a quest for alternative methods of disease intervention and optimization of growth promotion in commercial poultry farming. The use of DFM as an alternative approach has gained momentum in recent years. The advantages of easy application, pathogen reduction, immunomodulation, performance enhancement, and synthesis of antimicrobials and enzymes make DFM a viable alternative to antibiotics (Hong et al., 2005; Shin et al., 2008). In these experiments, we evaluated potential *Bacillus* spp.-based DFM that primarily reduced *Salmonella* incidence with additional beneficial effects on BW gain in both chicks and poults.

In experiment 1, 8 potential DFM candidates were chosen based on their ability to inhibit *Salmonella* in vitro and sporulate to high numbers (approximately 10^9 to 10^{11} spores/g) in a solid-state fermentation medium. This was important because higher yield directly translates to lesser feed inclusion rates at doses that are still efficacious in reducing *Salmonella* colonization. All of our isolates tested in vivo were of generally recognized as safe status (Table 1). The 2 most important characteristics of a DFM are pathogen reduction and improved BW gain. In these studies, reduction in the incidence or total *Salmonella* or both was the primary criterion for effective use as potential DFM candidates. In experiment 1, 5 of the 8 DFM candidates were able to reduce ST recovery in the crop of chicks, whereas 2 DFM candidates reduced ST recovery in the crop of poults (Table 3). In experiment 2, NP122 was able to decrease total cecal ST colony-forming units in both chicks and poults by approximately 1.5 log (Table 6). This is significant because ceca are the preliminary sites of colonization and a reduction in cecal *Salmonella* may result in lesser incidence of translocation to other organs and decrease the overall pathogen load in birds.

Prevalence of *Salmonella* in raw poultry products has always been a matter of concern. In a recent study, *Salmonella* incidence in raw poultry meat was at a significantly high rate of 60% and was therefore a cause of significant concern (Antunes et al., 2003). In an independent research study of raw meat products, chickens and turkeys were significantly contaminated by *Salmonella* compared with either pork or beef (Zhao et al., 2001). From a DFM perspective, many of the isolates evaluated in the present study showed promise in reducing *Salmonella* incidence in either chicks or poults but not both. However, NP122 was able to consistently reduce total *Salmonella* colony-forming units and caused

Table 6. Effect of NP122 on *Salmonella* Typhimurium (ST) cecal colony-forming units in broiler chicks and turkey poults at 11 d posthatch in experiment 2[1]

Treatment[2]	Chicks	Poults
ST only	2.37 ± 0.4[a]	2.05 ± 0.4[a]
ST + NP122	0.97 ± 0.3[b]	0.75 ± 0.3[b]

[a,b]Means within a column with different superscripts differ significantly ($P < 0.05$).

[1]Data expressed as $\log_{10}$ colony-forming unit counts of *Salmonella* in ceca samples (n = 20/treatment).

[2]See Table 1 for NP122 description.

Table 7. Effect of NP122 on recovery of *Salmonella* Typhimurium (ST) in broiler chicks and turkey poults at 11 d posthatch in experiment 2[1]

	Chicks		Poults	
Treatment[2]	Crop	Ceca	Crop	Ceca
ST only	80	100	40	100
ST + NP122	45*	95	35	95

[1]Data expressed as total percentage positive for *Salmonella* (n = 20/treatment).

[2]See Table 1 for NP122 description.

*Significantly different from control ($P < 0.05$).

lesser *Salmonella* recovery in both avian species tested, thereby demonstrating efficacy in multiple species of poultry. Even though these studies evaluated effects of DFM candidates on *Salmonella* incidence as early as 11 d posthatch, the results are promising and further studies will evaluate the efficacy of NP122 alone or in combination with other isolates in older birds.

Production parameters have seen enormous changes over the past 2 decades with increasing importance being given to growth promotion. The process of competitive exclusion of pathogens by DFM has been discussed extensively and may be applicable in the current situation (Barbosa et al., 2005). Studies involving *Bacillus* spp. specifically have seen growth benefits in turkey poults (Grimes et al., 2008), broilers (Vilà et al., 2009), and swine (Alexopoulos et al., 2004; Davis et al., 2008). In experiment 1, almost all DFM candidates tested helped improve BW gain. However, not all DFM candidates caused significant reduction in pathogen numbers, which was our primary objective. Therefore, it was important to select DFM candidates that enhanced pathogen reduction and demonstrated growth benefits. Even though BW gain was evaluated as a secondary character of DFM efficacy, it is important to observe that at all time points birds fed NP122 had higher BW gain when compared with birds not treated with NP122, irrespective of the presence or absence of a *Salmonella* challenge. Taken together, these studies provide preliminary evidence to demonstrate the efficacy of NP122 as a potential DFM candidate by reducing *Salmonella* colonization with additional benefits on BW gain in chicks and poults. Further studies will evaluate the potential of this candidate alone or in combination with other candidates to reduce *Salmonella* over an extended period of time.

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