

Research Notes

Evaluation of selected direct-fed microbial candidates on live performance and *Salmonella* reduction in commercial turkey brooding houses

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ABSTRACT As effective probiotic *Bacillus* isolates that can increase BW gain (BWG) are identified, they may offer advantages in terms of stability, cost, and feed application over probiotics limited to drinking water application. Additionally, an effective direct-fed microbial (DFM) may offer an effective alternative to antibiotic growth promoters. Previously, 4 *Bacillus* isolates were identified and evaluated in our laboratory as potential DFM candidates. These isolates were shown to significantly increase BWG as well as reduce recovery of *Salmonella* after experimental infection. In the first experiment, isolates PHL-MM65 (a *Bacillus laterosporus*) and PHL-NP122 (a *Bacillus subtilis*) were evaluated using poult raised under commercial conditions. After 7 d of conventional brooding, poult were tagged, weighed, and placed in 1 of 4 replicate pens for each treatment group [negative control, 0.019% nitarson, PHL-MM65 (10^6 spores/g of feed), or PHL-NP122 (10^6 spores/g of feed)] within the commercial turkey barn. At 23 d,

poult were weighed and BW was calculated. Treatment with PHL-NP122 (853 g) or nitarson (852 g) increased BW ($P \leq 0.05$) compared with control (784 g), whereas treatment with PHL-MM65 (794 g) did not significantly improve BW. Also on d 23 of the trial, ceca were aseptically removed from 10 poult per pen and cultured for recovery of *Salmonella*. Both *Bacillus* isolates PHL-NP122 and PHL-MM65 resulted in a significant reduction ($P \leq 0.05$) in the frequency of *Salmonella* by more than 25% compared with the controls. In a second experiment on a different farm, isolates PHL-NP122, PHL-RW33 (a *B. subtilis*), and PHL-B1 (a *Bacillus licheniformis*) were evaluated. None of the candidate *Bacillus* DFM or the group fed nitarson had significantly different BW or BWG than untreated control. These data suggest that isolate PHL-NP122, when added as a DFM to turkey diets, may increase BW gain as well as nitarson during the brooding phase of commercial turkey production.

Key words: direct-fed microbial, *Salmonella*, turkey, probiotic, *Bacillus*

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INTRODUCTION

The practice of using low-level antimicrobials as antibiotic growth promoters (AGP) has come under increasing pressure from consumers and government regulatory agencies in recent years as concerns over antibiotic resistance, antibiotic residues, arsenic residues, and environmental arsenic contamination continue to increase. An in-feed probiotic that provides similar increases in production parameters as those seen with an AGP may provide a viable alternative to AGP use in commercial poultry.

The use of beneficial bacteria in poultry dates back at least to when Nurmi and Rantala (1973) discovered

the concept of competitive exclusion by using gut homogenate from healthy adult chickens to protect chicks from experimental challenge with *Salmonella enterica* serovar Infantis. Different strains and cultures of beneficial bacteria administered to poultry and other livestock have been shown to increase production parameters such as weight gain and feed conversion (Zani et al., 1998; Hakkinen and Schneitz, 1999; Fritts et al., 2000; Guo et al., 2006; Vicente et al., 2007b) as well as reduce specific enteric pathogens of consequence to both humans and animals (Higgins et al., 2007; Newaj-Fyzul et al., 2007; Vicente et al., 2007a; Wolfenden et al., 2007; Davis et al., 2008).

Currently, most poultry feed in the United States is pelleted to increase feed consumption and BW gain (BWG; Choi et al., 1986). To steam-pellet feed, feed is heated to temperatures between 76.7 and 93.3°C (Cutlip et al., 2006). This process has been shown to reduce the presence of vegetative bacteria within the feed (Fu-

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ruta et al., 1980; Cox et al., 1983); thus, the addition of vegetative probiotic bacteria to feed before pelleting is problematic. Because most integrated poultry producers prefer to administer additives via the feed, this problem has been one of the primary inhibitors to the widespread use of probiotics.

Unlike many other types of probiotic bacteria that are delivered to the host animal as vegetative cells, *Bacillus* probiotics can be delivered as spores, the survival form of this genus of bacteria. Because they are stable and heat resistant (Nicholson, 2002; Setlow et al., 2006; Moeller et al., 2009), spores are ideally suited for in-feed application even in pelleted feeds. *Bacillus* have traditionally been thought of as soil-dwelling saprophytes; however, recent evidence suggests that *Bacillus* germinates within the gastrointestinal tract of poultry (Barbosa et al., 2005) and that these bacteria may actually make up a small population within the normal microflora of poultry (Lu et al., 2003). Additionally, *Bacillus* may increase the production parameters of poultry when incorporated into the diet as a direct-fed microbial (DFM; Fritts et al., 2000; Vilà et al., 2009). An in-feed probiotic or DFM that provides similar increases in production parameters as those seen with an AGP that can also lower the level of foodborne pathogens within the gut may offer a viable alternative to AGP use in commercial poultry.

Four strains of *Bacillus* were previously identified as potential probiotic or DFM candidates (Shivaramaiah et al., 2011). The objective of the present experiments was to evaluate the efficacy of these isolates as candidate DFM during the brooding phase of commercial turkey production.

MATERIALS AND METHODS

Candidate DFM Isolates

Shivaramaiah et al. (2011) isolated and evaluated *Bacillus* isolates for effect on growth rate and rate of recovery of *Salmonella* Typhimurium after experimental challenge. *Bacillus subtilis* isolates PHL-NP122 and PHL-RW33, *Bacillus licheniformis* isolate PHL-B1, and *Bacillus laterosporus* isolate PHL-MM65 were selected for further evaluation in commercial turkey brooding houses because of their performance in this study. These candidate *Bacillus* isolates were selected using the methods described by Wolfenden et al. (2010).

Experiment 1

Turkey poults were reared conventionally in commercial houses within brooder rings for 6 d. Prior to the removal of the brooder rings on d 7, a sample of 100 turkey poults was weighed and the mean BW and standard deviation were calculated. A total of 480 poults within 1 standard deviation of the sample mean were randomly assigned to groups (n = 120/group),

tagged, and placed into the block-randomized pens (1.5 m²) placed down the center aisle of the house (n = 30 poults/pen, 4 pens/group) similar to the procedure described by Torres-Rodriguez et al. (2007b). Each pen was equipped with a single manual feeder and an automatic poultry drinker. Poults were fed a commercial turkey starter diet with 0.019% nitarstone, a candidate *Bacillus* DFM PHL-NP122 or PHL-MM65 at the rate of 10⁶ spores/g of finished feed, or no supplementation (negative control; Tables 1 and 2). Both nitarstone, an organic arsenical used by some turkey producers, and the DFM candidates were fed continuously throughout the trial. Poults were weighed individually on d 1, 14, and 23 of the trial, and mean BW and BWG were used to evaluate the efficacy of the DFM in regards to production parameters.

On d 23, 10 poults per pen were killed by cervical dislocation at the farm. The ceca were then aseptically removed and transported on ice to the Poultry Health Laboratory at the University of Arkansas (Fayetteville). The cecal samples were macerated and diluted 1:4 with sterile saline and then enumerated using 10-fold dilutions and plate counting following overnight incubation at 37°C on brilliant green agar (catalog no. 228530, Becton Dickinson, Sparks, MD) containing 25 µg/mL of novobiocin (catalog no. N1628, Sigma, St. Louis, MO), MacConkey agar (catalog no. 212123, Becton Dickinson), and Rogosa SL agar (catalog no. 248020, Becton Dickinson) for enumeration of *Salmonella*, coliforms, and oxygen-tolerant lactic acid bacteria, respectively. Tetrathionate broth (catalog no. 210420, Becton Dickinson) was added to the cecal samples and incubated for 24 h for enrichment of *Salmonella*. The enriched samples were streaked onto brilliant green agar with 25 µg/mL of novobiocin and incubated overnight. The plates were evaluated for colonies characteristic of *Salmonella*, coliforms, or lactic acid bacteria on the appropriate selective media. This trial was conducted on a commercial farm and the integrator allowed only 10 poults per pen to be killed for this study. Because of this limitation, the *Salmonella* incidence data were pooled by group. All mortalities throughout the trial were necropsied by a qualified veterinarian. One pen each from the groups fed nitarstone and PHL-MM65 was removed from the statistical analysis because feeders were improperly adjusted for a 24- to 48-h period, resulting in a reduction of feed intake in those pens during that period. Poults were not cultured for *Salmonella* before the initiation of this trial because no reliable, nondestructive method of sampling individual poults exists. A positive cloacal swab provides confirmation that an individual poult is positive for *Salmonella*; however, the intermittent shedding of *Salmonella* by poultry leads to an unacceptable number of false negatives (Williams, 1978; Kotton et al., 2006). Given this and the random placement of poults, we felt that testing for *Salmonella* before the initiation of this trial would be of limited value.

Table 1. Effect of direct-fed microbial candidates or nitarsone in the ceca of commercial turkeys in experiment 1

Treatment ¹	BW (g)			Overall BW gain (g)	Poult positive for <i>Salmonella</i> in the ceca (%)	<i>Salmonella</i> cultured from ceca (log ₁₀ cfu)	Coliforms cultured from ceca (log ₁₀ cfu)	Lactic acid bacteria cultured from ceca (log ₁₀ cfu)
	d 1	d 14	d 23					
Control	125.9 ± 1.1	418.4 ± 13.8	784.2 ± 11.7 ^b	659.2 ± 10.8 ^b	48 ^a	1.92 ± 0.5 ^a	5.91 ± 0.3 ^b	7.99 ± 0.1
Nitarsone	125.9 ± 1.2	454.0 ± 1.3	852.4 ± 10.5 ^a	726.5 ± 11.6 ^a	33 ^{ab}	0.95 ± 0.20 ^{ab}	6.75 ± 0.1 ^a	7.94 ± 0.1
PHL-NP122	123.7 ± 2.0	454.8 ± 9.8	852.8 ± 10.1 ^a	727.9 ± 8.7 ^a	18 ^b	0.47 ± 0.2 ^b	6.10 ± 0.2 ^b	7.91 ± 0.5
PHL-MM65	125.6 ± 2.0	429.8 ± 1.4	793.9 ± 4.4 ^b	668.3 ± 5.4 ^b	23 ^b	0.89 ± 0.38 ^{ab}	5.78 ± 0.2 ^b	8.07 ± 0.4

^{a,b}Means within a column with different superscripts differ significantly ($P \leq 0.05$).

¹Four groups of poult (control, nitarsone, *Bacillus subtilis* isolate PHL-NP122, and *Bacillus laterosporus* isolate PHL-MM65; n = 120) were placed in block randomized pens (4 × 4 configuration) in the center of a commercial turkey brooder house for 23 d. Nitarsone was added to feed at the label inclusion rate of 0.019%, and direct-fed microbial candidates were fed at the rate of 10⁶ spores/g of feed.

Experiment 2

The experimental design of experiment 2 was similar to that of experiment 1, but experiment 2 was performed on a different farm and isolates PHL-RW33 and PHL-B1 were tested in place of isolate PHL-MM65. In this study a fifth group was added, necessitating an additional pen per block. The 5 groups evaluated in this trial were nonmedicated control, 0.019% nitarsone, or *Bacillus* candidates PHL-NP122, PHL-RW33, or PHL-B1 (each at the rate of ~10⁶ spores/g of feed). This experiment ended on d 30 when final weights were obtained. Bacteriological sampling was not conducted for this experiment because *Salmonella* was not detected in the ceca of any of the 20 poult sampled from the general population of the house on d 23 of the trial.

Statistical Analysis

All numerical data from these studies were subjected to ANOVA using JMP7 (JMP Software, Cary, NC), and partitioned treatment means at $P < 0.05$ indicated statistical significance. The percentage recovery of *Salmonella* was compared using the chi-squared test of independence, testing all possible group combinations to determine significance ($P \leq 0.05$) for these studies (Zar, 1984). As mentioned previously, the *Salmonella* incidence data were pooled by group.

RESULTS AND DISCUSSION

In the present study the experiments took place in commercial turkey brooding housing during a commercial production cycle to more adequately assess the efficacy of these DFM candidates. Each isolate in these experiments had previously been shown to increase BWG and decrease the recovery of *Salmonella* Typhimurium after experimental infection in preliminary laboratory trials (Shivaramaiah et al., 2011). These preliminary trials were conducted in environmentally controlled rooms on fresh bedding material. Access to these rooms was heavily restricted and strict biosecurity measures were followed. As such, both the level and scope of the microbial load to which the poult were exposed in these laboratory studies is undoubtedly much less than would typically be encountered by poultry reared under commercial conditions.

In experiment 1, BW was measured on d 1, 14, and 23. No differences were seen on d 1 or 14; however, the groups fed nitarsone or PHL-NP122 were heavier ($P \leq 0.05$) by d 23 (Table 1). These groups were 8.7% heavier than the nonmedicated group and 7.4% heavier than the group fed PHL-MM65. The overall BWG of the groups fed nitarsone and PHL-NP122 was also significantly greater ($P \leq 0.05$) than that of the group fed PHL-MM65 or the nonmedicated control (Table 1).

In experiment 1, the ceca were aseptically removed from 10 poult per pen to culture for *Salmonella*, coliforms, and lactic acid bacteria. Both groups fed the

Table 2. Effect of direct-fed microbial candidates or nitarsons on commercial turkeys in experiment 2

Treatment ¹	BW (g)				BW gain (g)	
	d 1	d 14	d 24	d 30	d 1–24	Overall
Control	134.2 ± 0.8	470.6 ± 9.9	896.6 ± 6.3	1,229.3 ± 8.3 ^{ab}	762.4 ± 6.3	1,095.5 ± 9.0 ^{ab}
Nitarsons	133.3 ± 1.2	472.7 ± 7.4	906.1 ± 8.6	1,277.2 ± 8.1 ^a	772.8 ± 9.5	1,144.1 ± 7.1 ^a
PHL-NP122	133.6 ± 0.4	456.8 ± 5.1	883.4 ± 6.9	1,224.9 ± 19.8 ^{ab}	749.9 ± 7.0	1,091.1 ± 19.6 ^{ab}
PHL-RW33	133.9 ± 0.3	461.6 ± 14.4	866.8 ± 19.2	1,190.3 ± 23.8 ^b	732.9 ± 18.1	1,056.3 ± 22.9 ^b
PHL-B1	133.7 ± 0.4	461.7 ± 3.3	895.6 ± 11.8	1,237.7 ± 20.0 ^{ab}	762.2 ± 10.9	1,104.5 ± 20.1 ^{ab}

^{a,b}Means within a column with different superscripts differ significantly ($P \leq 0.05$).

¹Five groups of poult (control, nitarsons, *Bacillus subtilis* isolates PHL-NP122 and PHL-RW33, and *Bacillus licheniformis* isolate PHL-B1; $n = 120$) were placed in block randomized pens (5 × 4 configuration) in the center of a commercial turkey brooder house for 30 d. Nitarsons was added to feed at the label inclusion rate of 0.019%, and direct-fed microbial candidates were fed at the rate of 10⁶ spores/g of feed.

candidate *Bacillus* DFM were found to have a lower ($P \leq 0.05$) percentage of *Salmonella* colonization compared with the negative controls, but only poult fed PHL-NP122 had significantly ($P \leq 0.05$) less *Salmonella* isolated (0.47 log₁₀ cfu/g) from the ceca compared with the nonmedicated controls (1.92 log₁₀ cfu/g; Table 1). Because this trial took place in commercial housing, the *Salmonella* infection seen in these poult was a naturally occurring infection and not an experimental challenge. The number of coliforms isolated from the ceca of the group treated with nitarsons was greater ($P \leq 0.05$) than the number isolated from all other groups. This increase, although statistically significant, did not negatively affect measured performance; the group fed nitarsons had greater BW and BW gain than the negative control (Table 1). No significant differences ($P \geq 0.05$) were found in the number of oxygen-tolerant lactic acid bacteria isolated between groups.

Experiment 2 was conducted on a different farm. Body weights were obtained on d 1, 14, 24, and 30. Weights were not different ($P \geq 0.05$) between groups during the first 24 d of this experiment (Table 2). By d 30, the group receiving nitarsons (1,277 g) was heavier than the group fed PHL-RW33 (1,190 g), but no significant differences ($P \geq 0.05$) were found between the other groups in either BW (1,229, 1,224, and 1,238 g for control, PHL-NP122, and PHL-B1, respectively) or BWG (1,095, 1,144, 1,091, 1,056, and 1,104 g for control, nitarsons, PHL-NP122, PHL-RW33, and PHL-B1, respectively).

In these experiments, isolate PHL-NP122 and 3 other isolates (PHL-MM65, PHL-RW33, and PHL-B1) were evaluated for their efficacy as potential DFM candidates. In experiment 1, isolate PHL-NP122 increased performance parameters while reducing the amount of *Salmonella* recovered (Table 1). In experiment 2, no significant differences ($P \geq 0.05$) in BW or BWG were observed between isolate PHL-NP122 and the control. It is interesting to note that PHL-NP122 performed similarly to nitarsons in both experiments, in which none of the other 3 isolates tested showed any significant differences, positive or negative, in BW or BWG compared with the untreated control group, although the percentage of poult positive for *Salmonella* was significantly ($P \leq 0.05$) reduced by isolate PHL-MM65.

The efficacy of AGP is widely accepted and the use of these agents is widespread in production animal agriculture in the United States. It is important to note that although AGP improve performance approximately 70% of the time in production animals, no measurable positive effects occur in almost one-third of applications (Rosen, 1995). The mode of action of AGP is not clearly defined, but studies in germ-free animals and the lack of systemic absorption of some antibiotics used as AGP point to direct effect on the microflora (Dibner and Richards, 2005). Torres-Rodriguez et al. (2007a) reported a similar success rate with the lactic acid bacteria-based probiotic FloraMax-B11 in commercial turkeys. FloraMax-B11 was administered to 60 commercial turkey flocks and BW was measured at the sale of each flock. The weights of the flocks from farms that historically ranked in the bottom 75% by the integrator were significantly increased ($P \leq 0.05$), whereas the weights of the flocks sold from the top 25% of farms were not significantly changed ($P \geq 0.05$; Torres-Rodriguez et al., 2007a). For both AGP and effective probiotics, little positive effect would be anticipated in the best-performing flocks. Neither the groups treated with a DFM candidate nor the group treated with nitarsons showed significant improvements in either BW or BWG compared with the nonmedicated control group in experiment 2. Therefore, the conditions in this experiment likely did not predispose the poult to an increase in BWG by either an AGP or DFM treatment. In contrast, both the group treated with nitarsons and the group fed PHL-NP122 were significantly heavier ($P \leq 0.05$) than the untreated control group in experiment 1. The control group poult in experiment 2 were 6.2 and 11.1% heavier ($P \leq 0.05$) than the poult from the control group in experiment 1 on d 0 and 14 of the experiment, respectively; this may indicate that the poult in experiment 2 were grown under better conditions and were not predisposed to a performance increase by an AGP or DFM.

The isolate PHL-NP122 has been shown to increase BW and BWG and to reduce colonization of *Salmonella* both in experiment 1 of the present study and in previous experiments by Shivaramaiah et al. (2011). Presently, we report that similarly to AGP and other probiotics, this isolate does not produce an increase

in BW and BWG under all conditions. Although the mechanism or mechanisms by which probiotics and AGP are able to increase production parameters are not well characterized, it is likely that environmental factors as well as the type and challenge level of pathogens present contribute to the success or failure of both AGP and probiotics or DFM under commercial conditions. Further evaluation to better assess the consistency of effect of isolate PHL-NP122 as a DFM under commercial conditions is warranted, but the evidence presented here suggests that this isolate could be used both as an alternative to AGP and to decrease the level of colonization of *Salmonella* in commercial turkeys.

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