

## Bacillus spores may enhance broiler performance

Since the introduction of *Bacillus subtilis* C-3102 spores as an alternative growth promoter in broiler chicken feeds in the U.S. about three years ago, several pen and field trials have been conducted that demonstrated improvements in broiler chicken bodyweight, feed conversion ratio and carcass yield.

By **DANNY HOOGE**

Bacterial spores, from the Greek word "sporos" meaning seed, are inactive resting or resistant forms produced within the body of the bacterium in response to nutritional limitation or high population density.

The gram-positive, non-pathogenic bacillus species continue to be extensively researched and are well characterized and widely utilized in many commercial applications. Spores of various bacillus species, including *B. subtilis*, *B. cereus* and *B. clausii*, are used as probiotics for animals and humans (Spinosa et al., 2000).

In terms of resistance to extreme environmental stresses, the bacterial spore represents the "pinnacle of evolution," being highly resistant to a wide variety of physical stresses such as wet heat, dry heat, ultra-violet radiation, gamma radiation, oxidizing agents, chemicals, vacuum pressure and ultrahigh hydrostatic pressure (Nicholson, 2002). This is primarily due to an armor-like, multilayered protein shell or spore coat. The *B. subtilis* spore cell "is metabolically dormant and as close to indestructible as any cell found on earth; nonetheless, the spore retains the ability to revive almost immediately when nutrient returns to the environment" (Driks, 2002).

L-alanine has been reported to have functional roles in the initiation of *B.*

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Measurement <sup>1</sup>	Negative control	+ <i>B. subtilis</i>
<b>Trial 1 (June-August)</b>		
Bodyweight, lb.	3.891	3.964 (+1.87) <sup>2</sup>
Feed/bodyweight (21-42 days)	2.015 <sup>a</sup>	1.949 <sup>b</sup> (-3.28)
Feed/bodyweight	1.809	1.798 (-0.61)
Mortality, %	2.88	3.17 (+10.07)
Salmonella (+)/total	46/46 <sup>a</sup>	17/48 <sup>b</sup> (-64.58)
APC, log <sub>10</sub> cfu/mL	4.34 <sup>a</sup>	4.17 <sup>b</sup> (-32.39) <sup>2</sup>
<i>E. coli</i> , log <sub>10</sub> cfu/mL	2.58	2.72 (+38.04) <sup>2</sup>
Coliforms (non- <i>E. coli</i> ), log <sub>10</sub> cfu/mL	2.37 <sup>a</sup>	2.12 <sup>b</sup> (-43.77) <sup>2</sup>
Campylobacter, log <sub>10</sub> cfu/mL	3.43 <sup>a</sup>	2.85 <sup>b</sup> (-73.70) <sup>2</sup>
<b>Trial 2 (September-October)</b>		
Bodyweight, lb.	4.336 <sup>b</sup>	4.546 <sup>a</sup> (+4.83)
Feed/bodyweight (21-42 days)	1.966 <sup>a</sup>	1.928 <sup>b</sup> (-1.93)
Feed/bodyweight	1.780	1.759 (-1.18)
Mortality, %	1.58	2.08 (+31.65)
Salmonella (+)/total	48/48 <sup>a</sup>	24/48 <sup>b</sup> (-50.00)
APC, log <sub>10</sub> cfu/mL	4.64 <sup>a</sup>	4.39 <sup>b</sup> (-43.77) <sup>2</sup>
<i>E. coli</i> , log <sub>10</sub> cfu/mL	2.40 <sup>a</sup>	2.12 <sup>b</sup> (-47.52) <sup>2</sup>
Coliforms (non- <i>E. coli</i> ), log <sub>10</sub> cfu/mL	1.95 <sup>a</sup>	1.35 <sup>b</sup> (-74.88) <sup>2</sup>
Campylobacter, log <sub>10</sub> cfu/mL	3.10	3.24(+38.04) <sup>2</sup>
<b>Combined trials<sup>1</sup></b>		
Bodyweight, lb.	4.142 <sup>b</sup>	4.250 <sup>a</sup> (+2.61)
Feed/bodyweight (21-42 days)	1.997 <sup>a</sup>	1.946 <sup>b</sup> (-2.55)
Feed/bodyweight	1.800	1.782 (-1.00)
Mortality, %	2.93	2.50 (-14.68)
Salmonella (+)/total	94/94 <sup>a</sup>	41/96 <sup>b</sup> (-57.29)
APC, log <sub>10</sub> cfu/mL	4.49 <sup>a</sup>	4.28 <sup>b</sup> (-38.34) <sup>2</sup>
<i>E. coli</i> , log <sub>10</sub> cfu/mL	2.49	2.41 (-16.82) <sup>2</sup>
Coliforms (non- <i>E. coli</i> ), log <sub>10</sub> cfu/mL	2.16 <sup>a</sup>	1.73 <sup>b</sup> (-62.85) <sup>2</sup>
Campylobacter, log <sub>10</sub> cfu/mL	3.26 <sup>a</sup>	3.05 <sup>b</sup> (-38.34) <sup>2</sup>

<sup>a,b</sup>Pairs of means within a row and with different letter superscripts differ (P < 0.05).  
<sup>1</sup>In trials 1 and 2, negative control had 12 pens of 50 males plus 12 pens of 50 females and double these pen numbers (24 plus 24) for *B. subtilis* treatment. For combined data, outliers were removed so means may not reflect simple average of trial 1 and 2 values.  
<sup>2</sup>Relative change (%) compared to negative control. Microbe counts in the last four categories are based on actual numbers (e.g., combined trials aerobic plate count (APC) 30,903 versus 19,055 for -16.82%) rather than on log<sub>10</sub> values.

*subtilis* spore germination (Wax et al., 1967).

Among the natural performance enhancers used to replace or reduce the levels of antibiotics in broiler chicken feeds is a commercial, direct-fed microbial product containing *B. subtilis* C-3102 spores. This commercial *B. subtilis* product has been the leading alternative growth promoter for broiler chickens in Japan for many years and was introduced into the U.S. market about three years ago.

The stable additive contains viable bacterial spores (272 billion colony forming units (cfu)/lb. or 1 x 10<sup>10</sup> cfu/g). With heat resistance to 194°F (90°C), *B. subtilis* C-3102 spores remain viable under typical steam-pelleting conditions used in the poul-

try feed industry. The low inclusion rate of 30 g pure spores per metric ton (27.16 g per ton) provides 3 x 10<sup>5</sup> cfu of viable spores/g feed.

The commercial product, containing calcium carbonate, rice hulls and *B. subtilis* spores, is used at a recommended rate of 1 lb. per ton of feed (0.05% level provides 0.003% pure spores). The *B. subtilis* direct-fed microbial product is classed as an approved, "Generally Recognized As Safe" (GRAS) feed ingredient by the Food & Drug Administration and the American Association of Feed Control Officials (36.14, p. 239, *Official Publication*, 2000).

Because *B. subtilis* is not a normal intestinal microorganism and is a strict aerobe (a saprophyte typically found

on compost and other decaying organic matter), it is not able to either grow or metabolize vigorously in the gut. By continuous feeding to poultry, however, a larger than usual number of the organism can exist and become active in the gut so that intestinal conditions can be influenced. *B. subtilis* C-3102 spores are compatible with most antibiotics and can generally be used in feeds alone or in combination with an antibiotic or antibiotic shuttle program.

Based on poultry and swine research and field trials with *B. subtilis* by Calpis Co. Ltd. and Quality Traders Inc. (Hajime Ishimaru, 2002, personal communication) in Japan, U.S. and Brazil, the following antimicrobials have been used successfully in combination with the spores: bacitracin, chlortetracycline, enramycin, noshieptide, oxytetracycline, tiamulin, tylosin, lincomycin, roxarsone, lasalocid, monensin, narasin, nicarbazine and salinomycin.

The purpose of this report is to review results of pen trials and commercial field trials using *B. subtilis* C-3102 spores in broiler chicken feeds to date and to present conclusions and recommendations.

### Broiler pen trials

Results of two similar 42-day broiler chicken pen trials conducted at the University of Arkansas on fresh litter are reported in Table 1 (Fritts et al., 2000). There were 12 replicate pens of males and 12 pens of females for the negative (antibiotic-free) control treatment and 24 replicate pens of males and 24 pens of females for the *B. subtilis* treatment group. Each replicate pen had 50 chicks initially. Mash diets were used in both experiments. The first trial was carried out in the summer (June 30-Aug. 11) with predominately hot weather, and the second trial was done in the autumn (Sept. 1-Oct. 13) with milder weather conditions. The U.S. Department of Agriculture-approved whole-bird prechill carcass rinses were done to measure microbial counts by treatments.

Live performance and prechill carcass microbiological results generally were similar in trials 1 and 2, with *Escherichia coli* and campylobacter counts being somewhat variable and differences non-significant within individual trials. In trial 2, the *B. subtilis* (30 g per ton) starter diet significantly improved 21-day bodyweight (not shown in Table 1; 690 versus 665 g) compared to the negative control diet. For combined trial results, there were significant improvements in 21-42 day feed conversion ratio, 42-day bodyweight and carcass microbes (fewer salmonella-positive birds, aerobic plate counts, non-*E.*

TABLES							
2. Summary of results from three pen trials on litter at a company research farm in the U.S. in 1998 and 1999 comparing negative control versus <i>B. subtilis</i> C-3102 spores-supplemented diets <sup>1</sup>							
Dietary treatment	Body-weight, lb.	Feed/bodyweight	Mortality %	-----Log <sub>10</sub> cfu/g-----			
				-Enterobacteriaceae- Feces	Litter	--Clostridium-- Feces	Litter
Trial 1 (June-July, 1998)							
Negative control	3.342	1.941	9.0	—	—	—	—
<i>B. subtilis</i>	3.485	1.914	8.3	—	—	—	—
Trial 2 (Sept.-Oct., 1998)							
Negative control	4.187	1.789	2.3	—	—	—	—
<i>B. subtilis</i>	4.281	1.768	2.6	—	—	—	—
Trial 3 (Sept.-Oct., 1999)							
Negative control	4.175	1.830	3.8	7.02	5.26	3.07	3.05
<i>B. subtilis</i>	4.275	1.798	3.9	6.47	4.45	2.40	2.86
Combined trials (3)							
Negative control	3.901 <sup>b</sup>	1.853 <sup>a</sup>	5.03	—	—	—	—
<i>B. subtilis</i>	4.014 <sup>a</sup>	1.827 <sup>b</sup>	4.93	—	—	—	—
Relative change <sup>2</sup>	+2.90	-1.40	-1.99				

<sup>a,b</sup>For combined trials, bodyweight means differ at P = 0.0184 and feed conversion ratio means differ at P = 0.0139 using Paired T-test with three trial values per treatment in each case. Probabilities for mortality were 0.7745 as percentages and 0.7343 as arcsine transformed data.

<sup>1</sup>There were eight observations (replicate litter pens) of straight-run broiler chickens per treatment mean in trials 1, 2 and 3, and this was doubled for combined trials means.

<sup>2</sup>Relative change (%) compared to negative control.

### 3. Avian Farms male broiler chicken live performance and processing results at 40 days of age as affected by dietary treatments (negative control, antibiotic control or *B. subtilis* C-3102 spores diets plus lactobacilli) in a Brazilian litter pen trial in summer (Albino et al., 2000)

Parameter	Negative control	+ Avilamycin, 10 (21 days), 5 ppm	+ <i>B. subtilis</i> <sup>2</sup>
Bodyweight gain, lb.	4.927	5.088 (+3.27) <sup>3</sup>	5.148 (+4.47) <sup>3</sup>
Feed, lb. per bodyweight, lb.	1.862	1.809 (-2.85)	1.841 (-1.13)
Mortality, %	3.33	0.83 (-75.1)	2.50 (-24.9)
Dressed carcass, lb. <sup>1</sup>	4.279	4.418 (+3.25)	4.425 (+3.40)
Whole breast, %	26.53	26.69 (+0.60)	26.92 (+1.47)
Breast meat, %	19.25	19.07 (-0.94)	19.51 (+1.35)
Leg, %	24.21	24.42 (+0.87)	24.29 (+0.33)
Abdominal fat, %	1.415	1.408 (-0.49)	1.235 (-12.72)

<sup>1</sup>Feathers, blood, feet, head and neck removed.

<sup>2</sup>*B. subtilis* C-3102 spores (1 x 10<sup>10</sup> cfu/g) at a level of 0.003% in feed and "one time" treatment with *L. reuteri* and *L. johnsonii* (6.6 x 10<sup>9</sup> and 3.3 x 10<sup>9</sup> cfu/g dry matter with dextrin, respectively) in 25 g/20 L unchlorinated drinking water at chick placement (1 g powdered milk added/20 L water). There were six observations (replicate pens of 20 birds each) per mean. Four males per replicate pen were selected to represent the unit average weight for processing.

<sup>3</sup>Relative change (%) compared to negative control.

*coli* coliforms and campylobacter).

The authors concluded that the addition of *B. subtilis* spores to feeds may improve the live performance of broilers in the absence of antibiotics and may contribute to on-farm pathogen reduction.

Three pen trials were conducted a company research farm in the U.S. in 1998 and 1999 to evaluate live performance and intestinal microflora alterations using negative control diets versus *B. subtilis* C-3102 supplemented diets (Table 2). Corn-soy-based feeds were in mash form in trials 1 and 2 and were pelleted in trial 3. For coccidiosis control, salinomycin was used in trials 1 and 2, and salinomycin plus roxarsone were used in trial 3. Statistical analysis was done using the three means for each of the two treatments for bodyweight, feed conversion ratio and mortality (paired T-test).

At an average of 41.0 days, signifi-

cant improvements were found in bodyweight (+2.90%) and feed conversion ratio (-1.40%) with *B. subtilis* diets versus positive controls. There was no significant difference among treatments in mortality. It was concluded that adding *B. subtilis* C-3102 spores to diets resulted in better weight and feed efficiency than the negative control diets.

Albino et al. (2000) conducted a 40-day summer pen trial in Brazil using male Avian Farms broiler chickens to compare the following dietary treatments: (1) negative control; (2) plus avilamycin (10 parts per million to 21 days, 5 ppm to 40 days), or (3) plus *B. subtilis* C-3102 spores (30 g/mt) with a one time lactobacillus water treatment at chick placement. Corn-soy-based mash feeds were used. Results are given in Table 3. No findings from statistical analyses were given, but numerical differences mirror other trials.

Table 4 shows the carcass, breast and abdominal fat percentages of broiler chickens from two 1991 pen trials at a research farm in southern Japan by a leading broiler company comparing positive (antibiotic) control and *B. subtilis* supplemented diets. Fresh litter was used for each placement as is traditional in Japan. For combined trials and sexes, carcass as a percentage of live weight was significantly increased by the addition of *B. subtilis* (+1.29% relative). Improvements due to the *B. subtilis* spores were approaching significance ( $P < 0.09$ ) for breast as a percent of carcass (+5.47% relative) and abdominal fat as a percent of carcass (-9.03% relative).

### Broiler field trial

A broiler chicken field trial was conducted in 1999 at a 12-house site, with six houses on each side of a center road, in the U.S. to compare positive (antibiotic) control and *B. subtilis* (0.003%) spore-supplemented diets for one flock on used litter. The antibiotic was bacitracin-md. Valid results were obtained from five control and five test product houses (Table 5). Although numerical improvements favoring the use of *B. subtilis* at 47.9 days of age were apparent under these commercial conditions, no statistically significant differences were observed between dietary treatments.

### Evaluating microflora changes

Broiler chicks grown in an isolation unit in Japan on fresh litter and inoculated with *Salmonella typhimurium* SU-27 by gavage at four days of age had significantly lower levels of this pathogen in their ceca at 14 days of age when fed a diet containing *B. subtilis* C-3102 spores than when given control feed (Figure 1).

A commercial paired house trial with Ross broiler chickens on fresh litter was conducted in Japan to evaluate the effects of dietary *B. subtilis* spores on microbial counts in fresh feces (Figure 2). The *B. subtilis* addition to feed significantly increased lactobacilli numbers at 14 days, with a similar pattern (though nonsignificant) at 49 days. This appears to indicate a mutually beneficial relationship between *B. subtilis* and lactobacillus species. Both salmonella and *Clostridium perfringens* counts were significantly decreased at 49 days by *B. subtilis* diets compared to negative control. Although not shown, enterobacteriaceae counts at 49 days for negative control and *B. subtilis* treatments were 7.06 and 6.64 log<sub>10</sub> cfu/g feces, respectively, nonsignificantly lower.

From three commercial broiler houses

TABLE

#### 4. Broiler chicken processing results from two pen trials at a company research farm in southern Japan in 1991 comparing negative control and *B. subtilis* C-3102 spores-supplemented diets

Trial; sex; parameter	Negative control	+ <i>B. subtilis</i> spores, 0.003%
Trial 1		
Male		
Carcass, % of live weight	78.9	80.1 (+1.52) <sup>2</sup>
Breast, % of carcass	20.0	20.3 (+1.50)
Abdominal fat, % of carcass	6.26	4.93 (-21.25)
Female		
Carcass, % of live weight	78.8	80.1 (+1.65)
Breast, % of carcass	20.7	21.6 (+4.35)
Abdominal fat, % of carcass	6.72	6.41 (-4.61)
Trial 2		
Male		
Carcass, % of live weight	77.5	78.1 (+0.77)
Breast, % of carcass	18.9	21.2 (+12.17)
Abdominal fat, % of carcass	6.81	6.45 (-5.29)
Female		
Carcass, % of live weight	75.7	76.6 (+1.19)
Breast, % of carcass	19.0	19.8 (+4.21)
Abdominal fat, % of carcass	8.91	8.32 (-6.62)
Combined trials (and sexes) <sup>1</sup>		
Carcass, % of live weight	77.725 <sup>b</sup>	78.725 <sup>a</sup> (+1.29)
Breast, % of carcass	19.650	20.725 (+5.47)
Abdominal fat, % of carcass	7.175	6.527 (-9.03)

<sup>1</sup>Means within a row and having a common letter superscript differ by Paired T-test ( $P < 0.05$ ). Actual probabilities using percentages and arcsin transformed percentages, respectively, were: carcass, 0.0080 and 0.0179; breast, 0.0872 and 0.0866, and abdominal fat, 0.0708 and 0.0660.

<sup>2</sup>Relative change (%) compared to negative control.

TABLE

#### 5. Results of a 1999 U.S. commercial field trial with positive (antibiotic) control versus *B. subtilis* C-3102 spores-supplemented diets for broiler chickens in 10 houses at the same site<sup>1</sup>

Dietary treatment; house number	Number of chicks placed	Age, days	Bodyweight, kg	Feed, kg/bodyweight, kg	Mortality, %
Antibiotic control					
House 1	31,500	48.0	4.65	—	6.42
House 2	29,565	48.0	4.78	—	4.92
House 3	31,550	47.5	5.23	—	4.56
House 4	31,500	48.0	5.01	—	4.76
House 5	31,619	48.0	4.69	—	4.67
<i>B. subtilis</i>					
House 8	33,565	48.0	4.70	—	5.18
House 9	31,550	47.5	5.03	—	4.33
House 10	31,500	48.0	4.95	—	4.72
House 11	31,619	48.0	4.89	—	5.54
House 12	31,550	48.0	4.92	2.075 <sup>3</sup>	4.53
Combined houses					
Antibiotic control	155,734	47.9	4.872	2.194	5.066
<i>B. subtilis</i>	159,784	47.9	4.898	[2.166] <sup>3</sup>	4.860
Relative change <sup>2</sup>	—	—	+0.53%	[-1.28%] <sup>3</sup>	-4.07%

<sup>1</sup>Due to a feed delivery mixup, results for house 6 (antibiotic control) were omitted. House 7 (*B. subtilis* treatment) was omitted as an outlier (Q-test,  $P < 0.10$  level; Dean and Dixon, 1951) based on bodyweight of 4.39 lb., with other five houses ranging from 4.70 to 5.03 lb. The antibiotic used in control feeds was bacitracin-md.

<sup>2</sup>Relative change (%) compared to positive (antibiotic) control. House means were analyzed using Unpaired T-test with  $n = 5$  per treatment to get combined houses results. Probabilities were: bodyweight,  $P = 0.1054$ ; mortality by percent,  $P = 0.2061$ , and using arcsine transformation,  $P = 0.1996$ .

<sup>3</sup>Overall feed consumption only was available by treatment (except for house 12 with 307,480 lb. feed/148,194 lb. chicken = feed conversion ratio of 2.075). Overall feed conversion ratio for *B. subtilis* houses was 2.111 (1,844,880 lb. feed/874,124 lb. chicken). With outlier house 7 removed (estimated ratio was 1.836 using actual house 12 as basis), other five houses had estimated feed conversion ratio of 2.166.

fed negative control or *B. subtilis* C-3102 diets (34 to 56 days or 17 to 56 days), fecal samples were evaluated for pathogens as indicated in Table 6, trial 1. Broilers were grown on fresh litter. In trial 1, the number of campylobacter species bacteria were significantly reduced in fecal samples in either situation when *B. subtilis* was used. Simi-

larly, the number of *C. perfringens* bacteria were significantly reduced when *B. subtilis* was added to broiler diets after 17 days of age compared to using negative control diets.

In trial 2 on fresh litter, comparable results were obtained for *C. perfringens* and enterobacteriaceae, whereas no campylobacter species were detected.

Salmonella species counts were similar by dietary treatment (2.97 versus 3.06 log<sub>10</sub> cfu/g for negative control versus treated), but the *B. subtilis* addition to diets significantly reduced the incidence of salmonella in broilers from 17/30 to 5/30 (P < 0.01).

The protective effect and exclusion of intestinal pathogens in laboratory isolation unit chick tests and broiler chicken field trials with *B. subtilis* C-3102 were attributed to an increased number of intestinal lactobacilli and decreased numbers of intestinal pathogens, specifically campylobacter, salmonella and enterobacteriaceae (Maruta et al., 1996).

### Modes of action

Based on a review of scientific literature, *B. subtilis* C-3102 spores in broiler chicken feeds may exert their beneficial effects by one or more of at least four mechanisms: (1) oxygen consumption — these aerobic microbes create a more favorable environment for benevolent anaerobic species; (2) enhanced proliferation of lactobacilli — which in turn produce lactic acid that helps control pathogenic bacteria such as *E. coli*, salmonella, clostridia and campylobacter; (3) enzyme production — through release of proteases (Benassi and Bonanni, 1965), beta-mannanase (el Helow and Khattab, 1996) and many other enzymes that these germinating and outgrowing spores assist in the food digestion process, and (4) enhanced immune responses — through activation of macrophages and natural killer cells and induction of serum alpha-interferon.

Murata (1993) found that endospores of the *B. subtilis* and ascospores of the yeast *Saccharomyces cerevisiae* contained almost all of the activities for the same enzymes as vegetative cells.

In examining mouse fecal microflora after dietary components and *B. subtilis* (natto) spores were delivered by intubation, Hosoi et al. (1999) found that viable spores did not affect fecal enterobacteriaceae or enterococcus species but did affect bacteroidaceae and lactobacillus species. Autoclaved (dead) spores did not alter fecal microflora. *In vitro* cultures of *Lactobacillus murinus* from mouse feces together with intact spores of *B. subtilis* (natto) under aerobic conditions as a mixed culture resulted in enhanced growth of *L. murinus*. This effect was evident only in media containing either sucrose, glucose, maltose or fructose (simple sugars) but not in media containing corn starch, soluble starch, or microcrystalline cellulose (complex carbohydrates).

It was concluded that some “metabolites” of *B. subtilis* (natto) produced during germination and/or outgrowth of

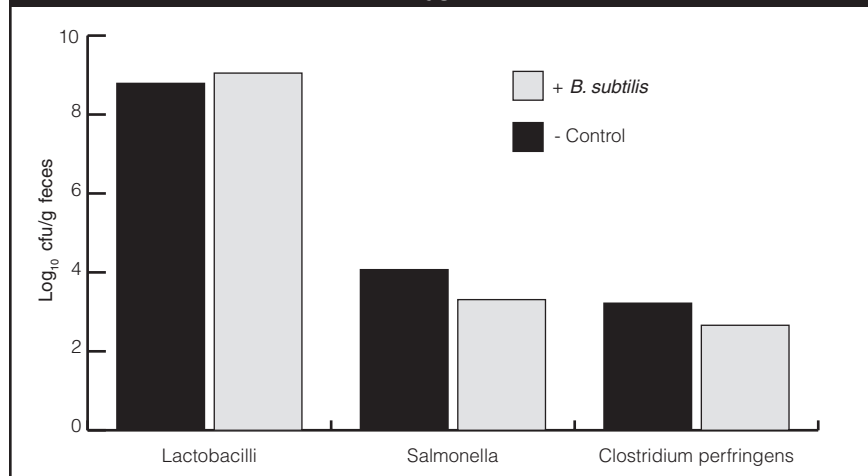
TABLE

6. Effect of *B. subtilis* C-3102 (30 g/mt) on pathogen counts in feces of Cobb broiler chickens under commercial conditions and fed treated diets from 34 to 56 or 17 to 56 days of age versus unsupplemented control feeds (0-56 days of age) in trial 1 and from 21 to 53 days of age in trial 2 (Maruta et al., 1996)<sup>1</sup>

Treatment	-----Bacteria/g feces (log <sub>10</sub> cfu/g)-----			
	<i>Campylobacter</i> spp.	<i>C. perfringens</i>	Enterobacteriaceae	<i>Salmonella</i> spp.
Trial 1				
Negative control	6.23 (50/50) <sup>2</sup>	3.15 (49/50)	7.21 (50/50)	—
+ <i>B. subtilis</i> (34-56 days)	4.20 <sup>c</sup> (20/50)	2.94 (43/50)	6.45 (50/50)	—
+ <i>B. subtilis</i> (17-56 days)	3.77 <sup>b</sup> (8/50)	2.57 <sup>c</sup> (40/50) <sup>a</sup>	6.88 (50/50)	—
Trial 2				
Negative control	ND <sup>3</sup> (0/30)	3.39 (28/30)	7.15 (30/30)	2.97 (17/30)
+ <i>B. subtilis</i> (21-53 days)	ND (0/30)	2.62 <sup>b</sup> (14/30) <sup>b</sup>	6.62 <sup>c</sup> (30/30)	3.06 (5/30) <sup>b</sup>

<sup>a,b,c</sup>The values differ significantly from the values of control; P < 0.05, <sup>b</sup>P < 0.01 or <sup>c</sup>P < 0.001. <sup>1</sup>Each mean is based on 2-3 fresh samples of feces (composite) per treatment in trial 1, and sampling not described in trial 2 but assumed to be similar. <sup>2</sup>Detected specimens/tested specimens. <sup>3</sup>None detected.

FIGURE



2. Effect of negative control or *B. subtilis* C-3102 (30 g/mt) diets on lactobacilli (14 days; P < 0.05), salmonella (49 days; P < 0.01; negative control 20/20 positive, *B. subtilis* group 10/20 positive, P < 0.05) and *C. perfringens* (49 days; P < 0.05) numbers in composite samples of fresh feces from commercial Chunky broiler chickens in a paired house trial in Japan (Maruta et al., 1996).

spores, interacting with monosaccharides or oligosaccharides, participated in the enhancement of growth of *L. murinus*.

La Ragione et al. (2001) used day-old, specific-pathogen White Leghorn chicks dosed with a suspension of *B. subtilis* PY79<sup>hr</sup> spores prior to challenge 24 hours later with *E. coli* O78:K80, a known virulent strain associated with avian colibacillosis.

A single oral inoculum of 2.5 x 10<sup>8</sup> spores was sufficient to suppress all aspects of *E. coli* O78:K80 infection. Colonization of deep organs was reduced by a factor of more than 2 log<sub>10</sub>, whereas colonization of the intestine, as measured by direct cloacal count, was reduced by more than 3 log<sub>10</sub>. Shedding of *E. coli* O78:K80, measured by semi-quantitative cloacal swabbing, was significantly reduced for the duration of

the 35-day experiment. *B. subtilis* persisted in the intestine although with decreasing numbers over the same period. Challenge with the same dose of pathogenic bacteria five days after pre-dosing with spores overcame any suppressive effect of the spores.

*B. subtilis* IP5832 shortens the duration of the carrier state in human patients with acute non-typhoid *Salmonella enteritidis* (Vukovic, 2001). Patients diagnosed with acute non-typhoid salmonella gastroenteritis accompanied by diarrhea for not longer than two days were given either placebo or *B. subtilis* spores (1 billion per capsule) three times daily (two capsules each time) for seven days. The *B. subtilis* treatment significantly reduced the number of diseased patients that remained carriers in the third week after completion of treatment and decreased the number positive find-

ings in stool cultures in the fourth week.

Coppi et al. (1985) observed that in human patients with stones-associated recurrent urinary tract infections, those treated with antibiotics followed by *B. subtilis* spores exhibited significant decreases in urine pH and in the frequency of infection episodes compared to results of those who received antibiotics alone.

Using *B. subtilis* A102 spores administered orally to mice, Kosaka et al. (1998) detected three-fold activation of murine macrophages and natural killer cells for two or three days, respectively, following treatment. The effect was similar for live or dead spores up to 0.1 g per mouse, decreasing at more than 0.15 g per mouse.

Similarly, Kuravtsev et al. (1996) reported that a single 1 billion cfu (in 0.1 mL cultural fluid) intraperitoneal or intravaginal dose of live cultures of *B. subtilis* to female mice stimulated migration, absorption and, especially, bactericidal activity of peritoneal exudate. Live bacilli stimulated *in vivo* induction and production of endogenous serum alpha-interferon induced *in vitro* by the Newcastle disease virus. The highest immune-stimulating effect of a single introduction of *B. subtilis* was achieved 66 hours later and had a tendency to gradually decrease.

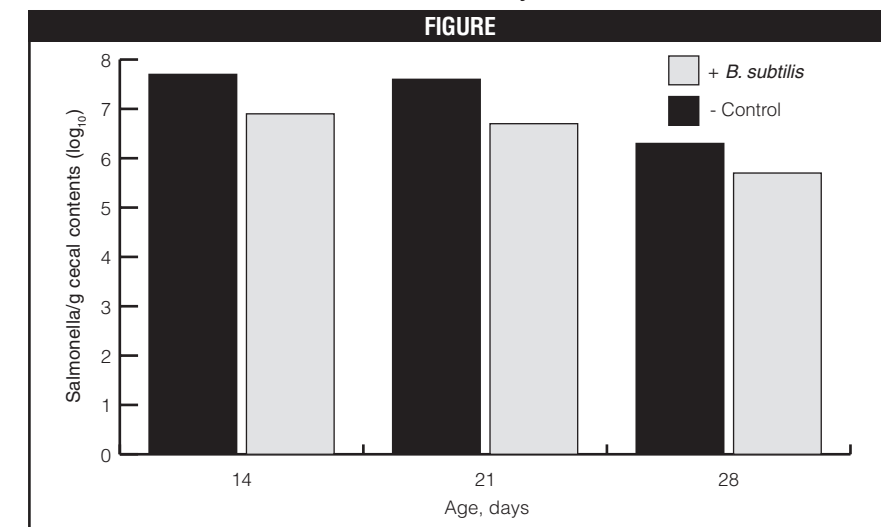
Fais et al. (1987) reported that *B. subtilis* spores, over a wide range of concentrations *in vivo* using peripheral mononuclear cells and activation antigens (MLR3, MLR4 and HLA-DR), induced lymphocyte activation that, at high spore concentrations, was quantitatively and qualitatively similar to the mitogens PHA and ConA, suggesting that T-cells may be involved in the response.

Ciprandi et al. (1986) concluded from *in vitro* work that the vegetative forms but not spores of *B. subtilis* enhanced mitogen (i.e., PHA and OKT3) induced the T-cell proliferation.

## Conclusions, recommendations

Since the introduction of *B. subtilis* C-3102 spores as an alternative growth promoter in broiler chicken feeds in the U.S. about three years ago, after having been used as the leading such product in Japan for many years, several pen and field trials have been conducted and results made available as reported herein. Significant ( $P < 0.05$ ) improvements have been obtained in broiler chicken bodyweight, feed conversion ratio and carcass yield.

Considering overall results from Tables 1, 2, 3 and 5, the relative magnitude of improvements compared to negative control (n = 4 values per treatment)



**1. Effect of dietary *B. subtilis* (0 or 30 g/mt) fed continuously on *S. typhimurium* SU-27 numbers in ceca of male broiler chicks after inoculation ( $1.6 \times 10^7$  cfu per bird) at four days of age (Maruta et al., 1996). Each mean is based on five birds per treatment and age. At 14 days, treatments differ ( $P < 0.05$ ).**

were: bodyweight, +2.77%; feed conversion ratio, -1.21%; mortality, -9.90%, and carcass yield, +1.29%. There were strong trends ( $P < 0.09$ ) for more breast yield (+1.29% relative) and less abdominal fat (-12.72%). Significant ( $P < 0.05$ ) reductions in the cecal, fecal and/or prechill carcass incidences of salmonella, *E. coli*, non-*E. coli* coliforms, campylobacter, *C. perfringens* and enterobacteriaceae were observed.

Based on a review of literature, the immunomodulatory and stabilizing effects of *B. subtilis* C-3102 viable spores on the intestinal microflora, especially the enhanced proliferation of lactobacilli, appear to be primarily responsible for broiler chicken live and processing benefits observed in several pen and commercial field trials.

The recommended use level of *B. subtilis* C-3102 is 0.003% pure spores or 0.05% as the commercial product containing the spores. *B. subtilis* C-3102 spores should be considered a viable alternative as a natural performance enhancer for broiler chickens.

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