Research Notes

Reduction of *Salmonella enterica* var. Enteritidis colonization and invasion by *Bacillus cereus* var. *toyoi* inclusion in poultry feeds

B. Vilà,*[1] A. Fontgibell,* I. Badiola,† E. Esteve-Garcia,* G. Jiménez,‡ M. Castillo,‡ and J. Brufau*

*Institut de Recerca i Tecnologia Agroalimentàries, Animal Nutrition, Mas de Bover, Ctra. Reus-El Morell km 3.8, 43120 Constantí, Spain; †Centre de Recerca en Sanitat Animal, Edifici V, Campus Universitari de Bellaterra, 08193 Cerdanyola del Valles, Spain; and ‡Rubinum Animal Health, Av. de la Llana no. 123, Pol. Ind. "La Llana," 08191 Rubí, Spain

ABSTRACT The effect of continuously feeding the probiotic microorganism Toyocerin to birds inoculated with *Salmonella* Enteritidis field-isolated strains on *Salmonella* Enteritidis prevalence, and performance variables were studied in 2 experiments. The experiments were performed with 1) broiler chickens in floor pens until slaughter 42 d of age, challenge was performed on d 3, 7, or 14 with 2×10^6 cfu per chick, and 2) Single Comb White Leghorn chickens in cages until 28 d of age, challenge was performed on d 7 with 10^8 cfu per chick. The inclusion of Toyocerin in feed of inoculated broiler chickens did significantly ($P < 0.05$) improve ADG (by 3.4 g), BW (by 141 g), and feed conversion ratio (by −0.060 kg/kg) at the end of the trial at 42 d compared with inoculated and untreated birds. At the end of the trial at 42 d, the slaughter age, 42% of untreated birds were still positive for *Salmonella*, whereas *Salmonella* was not detected in Toyocerin-treated birds. In Leghorn chickens, at 3 wk after inoculation (the end of the trial), only 38% of birds from the Toyocerin-treated groups were *Salmonella*-positive, whereas 63% of birds were still *Salmonella*-positive in the untreated control treatment. No significant differences were detected in performance variables in Leghorn chickens. The results of the present experiments indicate that feeding Toyocerin reduced the prevalence of *Salmonella* in poultry and in the case of broiler chickens also significantly improved performance variables at slaughter age.

Key words: probiotic microorganism, *Salmonella enterica* var. Enteritidis, broiler, Leghorn chick, *Bacillus toyoi*

2009 Poultry Science 88:975–979
doi:10.3382/ps.2008-00483

INTRODUCTION

Salmonella enterica var. Enteritidis is an important pathogen for the poultry industry because of its ability to infect chickens and hens, which increases the risk of *Salmonella* reaching the food chain by contaminated products (Humphrey, 2006). Gross pathological observations on naturally or experimentally *Salmonella* Enteritidis-infected poultry revealed that this microorganism may cause systemic infection in both chicks and laying hens, accompanied by prolonged fecal shedding (Suzuki, 1994).

Poultry products may pose a risk if contaminated with pathogenic microorganisms such as *Salmonella* and *Campylobacter*. To improve food safety, the industry is requested to decrease the level of contamination to zero or at least to acceptable levels (EFSA, 2007). Several intervention strategies have been applied starting at the breeding and production farm level through to the final product. Part of these intervention strategies are the use of probiotic microorganisms for prophylactic purposes.

Competitive exclusion by well-balanced native microflora can decrease the presence of *Salmonella* in optimal conditions. However, this balance within the gastrointestinal tract is challenged under commercial production when animals are subjected to stressful conditions such as hot weather and humidity, feed changes or imbalances, mycotoxin contamination, transportation, pathogens, molting, etc., thus increasing the risk of final product contamination. Feeding probiotic microorganisms continuously to animals has been found to maintain the beneficial intestinal microflora; this microflora regulation may serve 3 purposes: improve feed conversion and weight gain, improve the intestinal health and immune competence of the animals, and suppress foodborne pathogens such as *Salmonella* and *Campylobacter* species, which is important for the production of safe meat and meat products. Since Nurmi and Rantala (1973) first applied the concept of competitive exclusion in poultry to protect chickens against *Salmonella*

Received November 6, 2008.
Accepted January 15, 2009.
[1]Corresponding author: borja.vila@irta.cat

infection by inoculating them with microflora from adult birds, numerous studies have been performed on competitive exclusion (Weinack et al., 1982; Schneitz et al., 1998; Seo et al., 2000; Tellez et al., 2001; Nisbet, 2002). However, only a minority of microbes found in the chicken ceca have been isolated and identified to species level (Schneitz, 1998; Nisbet, 2002). Regulatory restrictions for probiotic microorganisms (SCAN, 2000) make this kind of product difficult to authorize. However, well-characterized microbial strains can also be successfully used to protect chickens or hens from foodborne pathogens (Kamata et al., 1990; Fritts et al., 2000; La Ragione and Woodward, 2003; Higgins et al., 2008). The present study examined the effect of continuously feeding the probiotic microorganism Toyocerin in broiler and Leghorn chickens inoculated with *Salmonella* Enteritidis field-isolated strains on postchallenge *Salmonella* Enteritidis prevalence in birds and performance variables.

MATERIALS AND METHODS

Birds

The experiments followed the European Union principals for animal care and experimentation. Experimental procedures were in agreement and approved by the Ethical Committee for Animal Care and Experimentation. Animals were obtained from commercial hatcheries. In experiment 1, four hundred 1-d-old Ross 308 sexed broiler chickens were used. Experiment 2 was carried out on 192 one-day-old Single Comb White Leghorn (**SCWL**) Hy-Line W98 male chickens.

A single basal diet, based on corn-soybean, was formulated to meet or exceed birds' requirements (NRC, 1994). Feed and water were offered for ad libitum consumption.

Challenge Microorganisms and Procedures

In experiment 1, the birds were inoculated at d 3, 7, or 14; an uninoculated group was also included. In experiment 2, birds were inoculated at d 7. Chickens were orally challenged with 1 mL of PBS suspension containing 2×10^6 (experiment 1) or 10^8 (experiment 2) cfu *Salmonella enterica* var. Enteritidis per milliliter (*Salmonella* Enteritidis, phage type 4, nalidixic acid-resistant strain, field isolate, CReSA S3146: experiment 1 or CReSA GN825: experiment 2).

Salmonella Recovery and Detection

Birds were killed by cervical dislocation and the ceca were collected aseptically. *Salmonella* Enteritidis recovery was conducted in accordance with the modified International Organization for Standardization standard. Briefly, tissue samples (approximately 20 g) were collected in a sterile plastic container with buffered peptone water (1.07228, Merck, Darmstadt, Germany) at a 1:10 ratio and homogenized. After an incubation period of 18 h at 37°C, 3 drops of the preenrichment broth, with a total volume of 0.1 mL, were inoculated on Rappaport-Vassiliadis (4649590922, Panreac Química SAU, Castellar del Vallès, Spain) semisolid media; the inoculated Rappaport-Vassiliadis plates were incubated at 42°C for 48 h. The GNI Vitek system (bioMérieux, Madrid, Spain) and PCR with specific primers were used to confirm the presence of *Salmonella*.

Probiotic Microorganism

The probiotic microorganism used was the product Toyocerin (powder feed additive EC no. 1701, containing 10^{10} viable spores of *Bacillus cereus* var. *toyoi* NCIMB 40112/CNCM I-1012 per gram, Rubinum Animal Health). The product was included at 0 or 100 mg/kg of feed in experiment 1 and at 0, 20, or 100 mg/kg of feed in experiment 2.

Experimental Designs

Experiment 1. Birds were raised in floor pens with supplemental heat until 42 d of age. Birds were distributed into 7 pens of males and 7 pens of females, at 29 chicks per pen. Design was a factorial one, being the factors studied the day of inoculation of *Salmonella* Enteritidis (3, 7, and 14 d, inoculated birds) and the inclusion of Toyocerin in the feed (levels: 0 mg/kg of feed = untreated birds and 100 mg/kg feed = treated birds), plus a negative control [uninoculated-untreated birds (**UU**)]. One pen of males and 1 pen of females were assigned to each treatment. Analysis for *Salmonella* presence was performed on 2 birds per pen at different days of trial. Body weight and feed consumption by replicate were checked at 10, 17, 28, and 42 d on trial; ADG, ADFI, and feed conversion ratio (**FCR**) were calculated thereafter. The European Production Efficiency Factor (EPEF) at 42 d was also calculated by replicate according to: EPEF = {[ADG (g)]/(FCR $\times$ 10)} $\times$ (100 $-$ % mortality).

Experiment 2. Birds were raised until 28 d of age in a Petersime brooder battery with supplemental heat. The design was randomized to study the inclusion of Toyocerin in the feed at levels 0, 20, and 100 mg/kg of feed. Each treatment was assigned to 8 cages of 8 birds. Analysis for *Salmonella* presence was performed on 1 bird per cage at weekly intervals. Body weight and feed consumption by replicate were checked at the end of the trial at 28 d; ADG, ADFI, and FCR were calculated thereafter.

Statistical Analysis

Data were analyzed according the design of each experiment. For performance variables, 1 cage or pen was considered the experimental unit, whereas for microbiological analysis, 1 bird was considered the experimental unit. Analysis was performed using the appropriate

Table 1. Summary results of performance variables (replicate = pen) for the whole trial period (1 to 42 d) from combining inoculated[1]-treated[2] (IT) or inoculated[1]-untreated (IU) groups of birds (Ross 308 broiler chickens) from experiment 1

Item	n	BW (g)	ADG (g)	FCR[3] (kg/kg)	EPEF[3]	Dead (%)
IT	6	2,164 ± 38.0	50.6 ± 0.91	1.831 ± 0.01352	256 ± 11.8	8.9 ± 3.51
IU	6	2,023 ± 39.1	47.3 ± 0.93	1.891 ± 0.01389	225 ± 12.2	11.7 ± 3.61
UU[4]	2	2,113 ± 65.9	49.4 ± 1.57	1.864 ± 0.02342	221 ± 20.5	18.5 ± 6.09
Estimated differences						
IT − IU		140.8 ± 54.51	3.35 ± 1.298	−0.06023 ± 0.01939	31.2 ± 16.97	−2.80 ± 5.039
P-value		*	*	*	0.116	0.599
IT − UU		51.5 ± 76.04	1.23 ± 1.811	−0.03327 ± 0.02704	34.6 ± 23.68	−9.59 ± 7.029
P-value		0.523	0.523	0.265	0.195	0.222
IU − UU		−89.3 ± 76.57	−2.13 ± 1.823	0.02696 ± 0.02723	3.4 ± 23.84	−6.79 ± 7.078
P-value		0.288	0.288	0.360	0.892	0.375

[1]Inoculated birds were given by gavage 1 mL of PBS suspension containing 2×10^6 cfu *Salmonella enterica* var. Enteritidis per milliliter (phage type 4, nalidixic acid-resistant strain, field isolate, CReSA S3146) at d 3, 7, or 14.

[2]Treated feeds included 100 mg of Toyocerin (feed additive EC no. 1701, containing 10^{10} viable spores of *Bacillus cereus* var. *toyoi* NCIMB 40112/CNCM I-1012 per gram) per kilogram of feed, providing 1×10^6 viable spores of *Bacillus cereus* var. *toyoi* per gram of feed.

[3]FCR = feed conversion ratio; EPEF = European Production Efficiency Factor = {[ADG (g)]/(FCR × 10)} × (100 − % mortality).

[4]UU = uninoculated, untreated.

*$P \leq 0.05$.

procedures of SAS System for Windows V8.02 (SAS, 2001). A significance level of $\alpha = 0.05$ was used.

RESULTS

Experiment 1

No significant interactions were detected between the main factors of the experiment (inoculation and feeding treatment) for any of the performance variables studied, except for global FCR. In this case, the inclusion of Toyocerin in feed significantly ($P < 0.05$) improved FCR (by 0.145 kg/kg) at the end of the trial at 42 d only when birds were inoculated at 3 d, but no significant differences were detected when birds were inoculated at 7 or 14 d (data not shown). The summary results of performance variables (replicate = pen) from combining inoculated-treated (**IT**) or inoculated-untreated (**IU**) groups are presented in Table 1. Performances variables of IU birds were not significantly different from UU birds, and therefore, differences in performance variables by challenge with *Salmonella* at the conditions of the experiment did not reach significance. However, the inclusion of Toyocerin in feed (IT) did significantly ($P < 0.05$) improve ADG (by 3.4 g), BW (by 141 g), and FCR (by −0.060 kg/kg) at the end of the trial at 42 d compared with IU birds.

Number of birds with presence of *Salmonella* is presented in Table 2. From inoculated birds, only the birds infected at 7 d and untreated had a delayed infection rate of 100% at 28 and 42 d, whereas birds also infected at 7 d but treated with Toyocerin had an infection rate of 50% at d 28, and of 0% at d 42. Only at 28 and 42 d were all groups sampled to detect the presence or absence of *Salmonella*; the Kruskal-Wallis test was performed to compare IU and IT groups at these sampling days. At 28 d, 58% of birds were positive for *Salmonella* in IU groups, whereas only 25% of birds were positive in IT birds. At the end of the trial at 42 d, that represented the slaughter age, 42% of IU birds were still

Table 2. Results of microbiological analysis from ceca for determination of the presence/absence of *Salmonella enterica* var. Enteritidis in experiment 1 (number of birds with presence of *Salmonella*/total number of birds sampled; replicate = bird; Kruskal-Wallis test)

Treatment	Day 3	Day 7	Day 10	Day 14	Day 17	Day 28	Day 42
$\text{Inoculated}_{\text{day 3}}$[1] untreated (IU)	—	1/4	0/4	—	—	2/4	0/4
$\text{Inoculated}_{\text{day 3}}$ treated[2] (IT)	—	1/4	2/4	—	—	1/4	0/4
$\text{Inoculated}_{\text{day 7}}$ untreated (IU)	—	—	0/4	2/4	—	4/4	4/4
$\text{Inoculated}_{\text{day 7}}$ treated (IT)	—	—	1/4	1/4	—	2/4	0/4
$\text{Inoculated}_{\text{day 14}}$ untreated (IU)	—	—	—	—	0/4	1/4	1/4
$\text{Inoculated}_{\text{day 14}}$ treated (IT)	—	—	—	—	0/4	0/4	0/4
Uninoculated untreated	0/4	0/4	0/4	0/4	0/4	0/4	0/4
IU						7/12	5/12
IT						3/12	0/12
P-value						0.105	*

[1]Inoculated birds were given by gavage 1 mL of PBS suspension containing 2×10^6 cfu *Salmonella enterica* var. Enteritidis per milliliter (phage type 4, nalidixic acid-resistant strain, field isolate, CReSA S3146).

[2]Treated feeds included 100 mg of Toyocerin (feed additive EC no. 1701, containing 10^{10} viable spores of *Bacillus cereus* var. *toyoi* NCIMB 40112/CNCM I-1012 per gram) per kilogram of feed, providing 1×10^6 viable spores of *Bacillus cereus* var. *toyoi* per gram of feed.

*$P \leq 0.05$.

positive for *Salmonella*, whereas *Salmonella* was not detected in IT birds.

These results suggest that Toyocerin-treated birds can arrive to the slaughter age of 42 d free of *Salmonella*, independently of the age of challenge, with significantly improved ADG (by 3.4 g), BW (by 141 g), and FCR (by −0.060 kg/kg), compared with birds IU, that still presented almost half of the birds positive to *Salmonella*.

Experiment 2

There were no significant differences among treatments on performance variables. The results were within expected ranges according to the trial conditions (White Leghorn male birds raised in cages feeding mash feeds): average values for the whole trial period (1 to 28 d) were BW at 28 d 364 and 371 g, ADG 11 and 12 g, FCR 2.030 and 1.987, for IU and IT groups, respectively, and 23 g of ADFI for both groups. Only 1 bird (from IU group) died during the trial.

Number of birds with presence of *Salmonella* is presented in Table 3. *Salmonella* was not detected before inoculation of the birds. Treatments including Toyocerin (IT) presented an earlier cleansing of *Salmonella* than the IU, with only 3 positive out of 8 birds at 3 wk postinoculation when Toyocerin was included at 20 or 100 mg/kg of feed, whereas 5 out of 8 birds were positive in the IU group. At 3 wk postinoculation (the end of the trial), only 38% of IT birds were *Salmonella*-positive, whereas 63% of IU birds were still *Salmonella*-positive.

DISCUSSION

In the trial with broiler chickens at slaughter age, IT birds had significantly better BW and ADG (7% improvement) and FCR (3% improvement) than IU birds. Santoso et al. (1995), van Wambeke and Peeters (1995), and Cavazzoni et al. (1998) found improvements in FCR but not in BW using *Bacillus subtilis*, *B. cereus* CIP 5832, or *Bacillus coagulans* CNCM I-1061, respectively. On the other hand, Fritts et al. (2000) concluded that the inclusion of *B. subtilis* C-3102 to broiler diets increased the final BW and FCR in the last half of the growing period of chickens, in accordance with our present work with Toyocerin. Fritts et al. (2000) did not inoculate birds with *Salmonella*; however, they detected 100% positive prechilled carcasses in untreated birds, whereas only 43% of the carcasses were positive for probiotic microorganism-treated birds. In our experiment, *Salmonella* was not detected at slaughter age in IT birds, whereas 42% of IU birds were positive. Kamata et al. (1990) also reported the absence of *Salmonella* at the end of the trial in *Salmonella* Typhimurium-inoculated broiler chickens when Toyocerin was continuously fed to birds. These authors found that Toyocerin encouraged proliferation of *Lactobacillus* spp., improving the balance of the intestinal microflora, digestion and absorption of ingested feed, and, consequently, weight gains and feed efficiency, improvements we also found in our experiment.

The experiment with SCWL chickens finished at 28 d of age, and although *Salmonella* was still present in IT birds at this time, only 38% of the birds were positive, whereas 62% of the IU birds were positive. In the trial with broiler chickens, the percentage of positive birds at 28 d on trial was very similar to the trial with SCWL chickens: 25 and 58% for IT and IU birds, respectively. No significant differences were found for BW or FCR in SCWL chickens by Toyocerin addition in feed; values were 371 vs. 364 g for BW and 1.989 vs. 2.030 kg/kg for FCR for IT and IU birds, respectively.

The benefits obtained by the inclusion of Toyocerin in poultry feeds in the experiments presented herein might be derived not only from the Nurmi concept but also from immune stimulation. Higgins et al. (2007) hypothesized that the innate immune system of chickens, specifically macrophages, played a role in reduction of *Salmonella* Enteritidis colonization with probiotic treatment. Khajarern and Ratanasethakul (1998) stated that when used continuously, *Bacillus toyoi* also

Table 3. Results of microbiological analysis from ceca for determination of the presence/absence of *Salmonella enterica* var. Enteritidis in experiment 2 (number of birds with presence of *Salmonella*/total number of birds sampled; replicate = bird; Kruskal-Wallis test)

Treatment	Day 7 (before inoculation)	Day 14	Day 21	Day 28
Inoculated[1] untreated (IU)	0/8	7/8	6/8	5/8
Inoculated treated$_{20}$[2] (IT)	0/8	8/8	7/8	3/8
Inoculated treated$_{100}$[2] (IT)	0/8	7/8	7/8	3/8
IU	0/8	7/8	6/8	5/8
IT	0/16	15/16	14/16	6/16
P-value	1	0.609	0.448	0.257

[1]Inoculated birds were given by gavage of 1 mL of PBS suspension containing 1×10^8 cfu *Salmonella enterica* var. Enteritidis per milliliter (phage type 4, nalidixic acid-resistant strain, field isolate, CReSA GN825) at 7 d of age.

[2]Treated feeds included 20 and 100 mg of Toyocerin (feed additive EC no. 1701, containing 10^{10} viable spores of *Bacillus cereus* var. *toyoi* NCIMB 40112/CNCM I-1012 per gram) per kilogram of feed, providing 2×10^5 and 1×10^6 viable spores of *Bacillus cereus* var. *toyoi* per gram of feed.

served to reinforce the nonspecific immune system of birds. Khaksefidi and Ghoorchi (2006) found positive effects on performance and the immune system of broiler chicks fed probiotic containing *B. subtilis*.

Adding a probiotic microorganism becomes a preventive measure against any detrimental effect on performance originated through the intestinal flora, and it might also reduce the incidence of *Salmonella*-contaminated poultry products by decreasing the incidence of the pathogen at the farm level. The results of the present experiments indicate that feeding Toyocerin reduced the prevalence of *Salmonella* in poultry and, in the case of broiler chickens, also significantly improved the performance variables at slaughter age.

REFERENCES

Cavazzoni, V., A. Adami, and C. Castrovilli. 1998. Performance of broiler chickens supplemented with *Bacillus coagulans* as probiotic. Br. Poult. Sci. 39:526–529.

EFSA. 2007. The Community Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents, Antimicrobial Resistance and Foodborne Outbreaks in the European Union in 2006. Eur. Food Saf. Authority, Parma, Italy.

Fritts, C. A., J. H. Kersey, M. A. Motl, E. C. Kroger, F. Yan, J. Si, Q. Jiang, M. M. Campos, A. L. Waldroup, and P. W. Waldroup. 2000. *Bacillus subtilis* C-3102 (Calsporin) improves live performance and microbiological status of broiler chickens. J. Appl. Poult. Res. 9:149–155.

Higgins, S. E., G. F. Erf, J. P. Higgins, S. N. Henderson, A. D. Wolfenden, G. Gaona-Ramirez, and B. M. Hargis. 2007. Effect of probiotic treatment in broiler chicks on intestinal macrophage numbers and phagocytosis of *Salmonella* Enteritidis by abdominal exudate cells. Poult. Sci. 86:2315–2321.

Higgins, S. E., J. P. Higgins, A. D. Wolfenden, S. N. Henderson, A. Torres-Rodriguez, G. Tellez, and B. Hargis. 2008. Evaluation of a *Lactobacillus*-based probiotic culture for the reduction of *Salmonella* Enteritidis in neonatal broiler chicks. Poult. Sci. 87:27–31.

Humphrey, T. 2006. Are happy chickens safer chickens? Poultry welfare and disease susceptibility. Br. Poult. Sci. 47:379–391.

Kamata, S. I., N. Kakiichi, and K. Uchida. 1990. Effect of continuous Toyocerin administration in the feed of chicken experimentally infected with *Salmonella* Typhimurium. Anim. Husbandry 44:385–388.

Khajarern, J., and C. Ratanasethakul. 1998. Probiotics in breeder feed. World Poult. 14:18–19.

Khaksefidi, A., and T. Ghoorchi. 2006. Effect of probiotic on performance and immunocompetence in broiler chicks. Jpn. Poult. Sci. 43:296–300.

La Ragione, R. M., and M. J. Woodward. 2003. Competitive exclusion by *Bacillus subtilis* spores of *Salmonella enterica* serotype Enteritidis and *Clostridium perfringens* in young chickens. Vet. Microbiol. 94:245–256.

Nisbet, D. 2002. Defined competitive exclusion cultures in the prevention of enteropathogen colonisation in poultry and swine. Antonie Van Leeuwenhoek 81:481–486.

NRC. 1994. Nutrient Requirements of Poultry. 9th rev. ed. Natl. Acad. Press, Washington DC.

Nurmi, E., and M. Rantala. 1973. New aspects of *Salmonella* infection in broiler production. Nature 241:210–211.

Santoso, U., K. Tanaka, and S. Ohtani. 1995. Effect of dried *Bacillus subtilis* culture on growth, body composition and hepatic lipogenic enzyme activity in female broiler chicks. Br. J. Nutr. 74:523–529.

SAS. 2001. SAS System for Windows V.8.02. SAS Inst. Inc., Cary, NC.

SCAN. 2000. Report of the Scientific Committee on Animal Nutrition on the assessment under directive 87/153/EEC of the efficacy of micro-organisms used as feed additives (expressed on 18 February 2000). Sci. Comm. Anim. Nutr., Brussels, Belgium.

Schneitz, C. 1998. Competitive exclusion of *Salmonella*: Defined or undefined products. Poult. Int. 1998:18–20.

Schneitz, C., T. Kiiskinen, V. Toivonen, and M. Näsi. 1998. Effect of Broilact® on the physicochemical conditions and nutrient digestibility in the gastrointestinal tract of broilers. Poult. Sci. 77:426–432.

Seo, K. H., P. S. Holt, R. K. Gast, and C. L. Hofacre. 2000. Combined effect of antibiotic and competitive exclusion treatment on *Salmonella* Enteritidis fecal shedding in molted laying hens. J. Food Prot. 63:545–554.

Suzuki, S. 1994. Pathogenicity of *Salmonella* Enteritidis in poultry. Int. J. Food Microbiol. 21:89–105.

Tellez, G., V. M. Petrone, M. Escorcia, T. Y. Morishita, C. W. Cobb, and L. Villaseñor. 2001. Evaluation of avian-specific probiotic and *Salmonella* Enteritidis-, *Salmonella* Typhimurium-, and *Salmonella* Heidelberg-specific antibodies on cecal colonization and organ invasion of *Salmonella* Enteritidis in broilers. J. Food Prot. 64:287–291.

van Wambeke, F., and J. Peeters. 1995. The effect of Paciflor® on the performances, carcass composition and caecal bacterial numbers of broilers. Arch. Geflügelkd. 59:125–129.

Weinack, O. M., G. H. Snoeyenbos, C. F. Smyser, and A. S. Soerjadi. 1982. Reciprocal competitive exclusion of *Salmonella* and *Escherichia coli* by native intestinal microflora of the chicken and turkey. Avian Dis. 26:585–595.