The Use of Competitive Exclusion in Broilers to Reduce the Level of *Salmonella* Contamination on the Farm and at the Processing Plant

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ABSTRACT The effect of a competitive exclusion (CE) product, Broilact®, on *Salmonella* contamination of broiler chickens was studied on the farm and at the processing plant. In the first part of the study, two flocks per week, a CE-treated and an untreated control flock, were placed in similar broiler houses. The CE treatment was administered in the hatchery using a modified spray vaccination cabinet. *Salmonella* was analyzed from the paper pads of the transport boxes on arrival at the farm and from fecal samples taken 2 wk before slaughter. The results of *Salmonella* sampling were received for 67 flocks. The other 141 flocks of the company that were reared during the trial period were also sampled for *Salmonella* and the results were compared to those of treatment and control groups. Broiler performance, including mortality, weight, and feed conversion, was recorded for the trial flocks. In the second part of the study, *Salmonella* contamination of neck skin samples taken at the processing plant from 18 CE-treated and 28 control flocks was compared. The Broilact®-treatment significantly reduced *Salmonella* contamination both on the farm and at the processing plant. At the level of the farm, the percentage of *Salmonella*-positive flocks was essentially the same in the control flocks and in other flocks reared during the trial period. An improvement in broiler performance was indicated, although the difference was not significant.

(*Key words*: poultry, *Salmonella*, farm hygiene, slaughter hygiene, field trial)

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INTRODUCTION

It was first shown by Nurmi and Rantala (1973) that mature intestinal flora effectively protect newly hatched chicks against colonization by *Salmonella* bacteria. The use of competitive exclusion (CE) involves giving the day-old chick normal intestinal bacterial flora derived from the adult chicken. Without normal flora, the chicks are vulnerable to just a few *Salmonella* cells (Seuna 1979). Broilact® is a commercial CE product based on Nurmi's research. It has been shown to protect birds against several *Salmonella* serotypes, including both invasive and noninvasive strains (Mead *et al*., 1989; Nuotio *et al*., 1992; Schneitz, 1992).

Effective control of processing hygiene will greatly depend on the *Salmonella* status of the flocks entering the processing plant (Mead, 1993; Salvat *et al*., 1993). Although cross-contamination of *Salmonella*-free carcasses is likely to occur during slaughter, a reduction in the number of *Salmonella*-positive carcasses at the end of the process should be possible by lessening the *Salmonella* challenge introduced into the slaughterhouse.

Because of the role of normal stabilized intestinal flora in preventing the colonization of harmful bacteria, as well as in enhancing the ingestion of feed, the use of CE should have a positive effect on broiler performance. Under controlled conditions, Broilact® has been shown to reduce mortality (Elwinger *et al*., 1992).

The efficacy of Broilact® in controlling *Salmonella* has been shown in a number of laboratory studies, but only few field trials have been published (Johnson, 1992; Wierup *et al*., 1992) none of which made use of the present freeze-dried form of the product. The purpose of this study was to evaluate the efficacy of CE in reducing *Salmonella* contamination of broiler chicks on the farm and at the processing plant, and to study its general effect on performance.

MATERIAL AND METHODS

Rearing and Slaughtering of the Flocks

Both trials were conducted at a poultry integrator in France. All broilers came from the same hatchery. A special crossbreed was used, with a light slaughter weight and good health status. The chicks were placed as hatched, 50% males and 50% females. The broiler houses were provided with similar nipple drinkers and feeding systems, brooder heating, and continuous light. Straw was used as litter material. The average number of birds in a flock was 22, 000. The chicks were fed a granulated feed

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made by the company feedmill. No growth promoter was used. The coccidiostats used in the starter, grower, and finisher diets were the same in the CE-treated and control houses, but were alternated according to a shuttle program. Coccidiostats included in the program during the trial period were monensin, salinomycine, maduramicin, halofuginone, nicarbacin, and diclazuril.

Vaccinations against Marek's disease and infectious bronchitis (IB) were given to the chicks in the hatchery before the CE treatment. No flocks were treated with antimicrobials.

The flocks were slaughtered at the age of 58 d at the same processing plant. The CE-treated flocks were processed first on a given day, or, alternatively, only CE-treated flocks were processed on a given day, to prevent cross-contamination from untreated flocks. The total number of flocks processed during a day ranged from one to three.

Competitive Exclusion Treatment

The paired treatment and control flocks were placed in their houses during the same week. The chicks assigned to the paired treatment and control houses originated from the same breeder flocks. The number of CE-treated and control birds was 615,000 and 658,000, respectively.

The dose of freeze-dried Broilact®[1] was 1 mg per chick. Broilact® was suspended in bottled spring water to obtain a volume of 0.2 mL per chick. The solution was administered to newly hatched chicks in the hatchery by droplet application. An automatic modified IB-vaccination cabinet was used, with constant pressure and two nozzles suitable for coarse spray.

Sampling of Salmonella

Altogether 34 CE-treated and 34 control flocks were sampled on the farm. In addition, *Salmonella* sampling was carried out in another 141 broiler flocks, representing the rest of the production of the company during the trial period. Five paper pads per flock were randomly taken from the chick delivery boxes on arrival at the farm. The five samples were pooled for qualitative *Salmonella* analysis. At the age of 45 d, 10 fecal droppings per flock were randomly collected from the litter and pooled for analysis. The total number of individual paper-pad and fecal samples was 340 and 680, respectively. In the second part of the study, 18 CE-treated and 28 untreated control flocks were sampled in the processing plant by taking swab samples of neck skin. The flocks were not the same as those used in the farm contamination study. Five samples of neck skin were randomly taken from each CE-treated flock after evisceration and analyzed individually. The flocks were processed either first of the day or during a day when only CE-treated flocks were slaughtered, to prevent cross-contamination from untreated flocks. Three samples of neck skin were taken from each control flock after wrapping and analyzed individually. The total number of individual samples in the treated and control flocks was 90 and 84, respectively.

Methods of Analysis

All *Salmonella* samples were analyzed in the same laboratory. Qualitative analysis for *Salmonella* was started with pre-enrichment in buffered peptone water,[2] which was incubated at 37 C for 24 h *Salmonella*-positive samples were identified using Oxoid Salmonella Rapid Test.[2] Positive samples were plated on Brilliant Green Agar[2] and modified X.L.T.4 medium[3] followed by incubation at 37 C for 24 h. Typical colonies were tested by agglutination using Pasteur institute sera.[4]

Broiler Performance

Comparison of performance was carried out in the same 34 CE-treated and 34 control flocks as those used in the *Salmonella* contamination study on the farm. Performance indicators included average live weight (ALW), total relative farm mortality (mortality%), and the feed conversion rate (FCR). The following formulas were used in calculating the indicators:

$$\text{Mortality\%} = \frac{\text{Number placed} - \text{Number slaughtered}}{\text{Number placed}} \times 100$$

$$\text{Weight (ALW)} = \frac{\text{Total weight of flock at arrival in processing plant}}{\text{Number of broilers processed}}$$

$$\text{FCR caught} = \frac{\text{Feed consumed}}{\text{Total weight of flock at arrival in processing plant}}$$

$$\text{FCR processed} = \frac{\text{Feed consumed}}{\text{Total weight of flock at arrival in processing plant} - \text{Weight of carcasses condemned}}$$

Statistical Analysis

Statistical analyses were carried out using SAS® 6.11.[5] Fisher's exact test was used to compare the percentage of *Salmonella*-positive flocks in the treatment and control groups and the χ^2 test to compare the proportion of

[1]Orion Corp. Animal Health, 20101 Turki, Finland.
[2]Unipath Ltd., Basingstoke, Hampshire, RG24 8PW, UK.
[3]AES Laboratorie, 35270 Combourg, France.
[4]Diagnostics Pasteur, 92430 Marnes-la-Coquette, France.
[5]SAS Institute Inc., Cary, NC 27513.

TABLE 1. Proportion of *Salmonella*-positive flocks in competitive exclusion treated and untreated control group on arrival at the farm and at the age of 45 d

Treatment	Number of flocks	*Salmonella*-positive flocks	
		At day old	At 45 d old
		(%)	
Broilact®	34	13[a] (4/32)	6[b] (2/34)
Control	34	25[a] (8/32)	42[a] (14/33)
Other production[1]	141	14[a] (20/140)	35[a] (50/141)

[a,b]Values within a column with no common superscript differ significantly ($P < 0.05$). Other flocks of the company reared during the trial period.

Salmonella-positive neck skin samples. Statistical analysis on performance indicators was carried out using the two-sample *t* test.

RESULTS

Effect of CE Treatment on Salmonella *Contamination on the Farm*

Salmonella results were obtained for 34 CE-treated and 33 control flocks. The fecal samples for one control flock were not received in the laboratory. Six percent of the CE-treated flocks (2 flocks of 34) and 42% of the control flocks (14 flocks of 33) were positive for *Salmonella* (Table 1). The difference was significant ($P = 0.0005$). Of the other 141 flocks reared during the trial period, 35% (50 flocks of 141) were positive; the proportion did not differ from the control group ($P = 0.548$).

On the average 16% of all the flocks arriving at the farm were *Salmonella*-positive according to the paper pad samples (Table 1). The proportion of positive flocks was 25% in the control group and 13% in the CE-treated group. The difference was not significant ($P = 0.337$). Fourteen percent of the other 141 flocks were contaminated; this percentage did not differ from the control group ($P = 0.185$).

All four flocks in the CE-treated group that tested positive on arrival at the farm tested negative when fecal samples were taken at the age of 45 d. Of the eight control flocks positive on arrival, four were still positive at this age. Of the 141 flocks outside the trial, 14 out of the 20 positives also remained positive.

Three CE-treated and nine control flocks that tested negative on arrival at the farm were positive at the age of 45 d. *Salmonella* samples were regularly taken from the breeder houses, the hatchers and feed. During the trial period, *Salmonella* was detected in three feed samples and in four swab samples taken from hatcher walls.

Broiler Performance

The performance data of six CE-treated and four control flocks were excluded because of the adverse effect of a heat wave. The performance of the CE-treated group, measured in terms of mortality, average live weight (ALW), and feed conversion rate (FCR), was not significantly better than performance of controls (Table 2).

Effect of CE Treatment on Salmonella *Contamination in the Processing Plant*

Neck skin samples were taken after evisceration from 18 CE-treated flocks and after wrapping from 28 untreated control flocks. The percentage of *Salmonella*-positive neck skin samples in the CE-treated group was significantly lower than in the control group ($P = 0.021$) (Table 3).

DISCUSSION

European Council directive 92/117/EEC concerns measures for protection against specified zoonoses and specified zoonotic agents in animals and products of animal origin, and is designed to prevent outbreaks of foodborne infections and intoxications. National *Salmonella* control programs based on this directive typically concentrate on excluding two invasive serotypes regarded as the most hazardous, *Salmonella enteritidis* and *Salmonella typhimurium*. However, the presence of any *Salmonella* serotype in production represents a consumer risk and is an indicator for the occurrence of more pathogenic serotypes.

Competitive exclusion treatment clearly reduced the number of flocks infected with *Salmonella* at the age of 45 d and at slaughter. The administration of Broilact® in the hatchery by the modified vaccination cabinet was shown to be an effective and safe means of treating commercial broilers. There were no adverse effects related either to the treatment material itself or to the wetting of the chicks, although the volume of fluid was increased by vaccines that were also sprayed in the

TABLE 2. Effect of competitive exclusion treatment on broiler performance

Treatment	Number of flocks	Age	Farm mortality ± SD	Average live weight ± SD	Feed conversion rate ± SD	
					Caught	Processed
		(d)	(%)	(kg)	(g:g)	
Broilact®	28	58	0.95 ± 1.06	2.102 ± 0.09	2.204 ± 0.062	2.208 ± 0.062
Control	30	58	1.04 ± 1.52	2.090 ± 0.08	2.212 ± 0.054	2.215 ± 0.054

TABLE 3. Proportion of *Salmonella*-positive neck skin samples in competitive exclusion treated and untreated control flocks at slaughter

Treatment	Number of flocks	Number of neck skin samples/flock	Total number of neck skin samples	Proportion of *Salmonella*-positive neck skin samples
				(%)
Broilact®	18	5	90	44 (49/90)[b]
Control	28	3	84	62 (52/84)[a]

[a,b]Figures within a column with no common superscript differ significantly ($P < 0.05$).

hatchery. The possible contribution of wetting to an increased susceptibility of chicks to infections has occasionally arisen in discussions in the field.

On average, 16% of all flocks were *Salmonella*-positive on arrival at the farm. It has been assumed that CE flora does not result in a significant reduction of *Salmonella* if the chick is already infected before treatment. However, in this trial all the flocks in the CE-treated group that were positive for *Salmonella* on arrival at the farm tested negative at the age of 45 d, whereas 50% of the correspondingly positive control flocks were still positive at the same age.

In both groups there were flocks that tested negative on arrival at the farm, but were positive at the age of 45 d. During rearing the birds may get infected through many routes, including environment, feed, and water (Franco and Williams, 1991). Occasional contamination of the feed with *Salmonella* was one of the elements increasing the challenge during rearing of the trial flocks. In addition, it is possible that the sampling method left a low level infection unnoticed at day-old and it spread among the broilers on the farm resulting in a positive test at the age of 45 d. Eleven percent of CE-treated and 38% of control flocks that tested negative on arrival were contaminated at the end of the rearing period.

Mortality was low during the trial period and no antibiotic treatments were necessary. No growth promoter was used in the production. Certain antibacterials may damage the CE flora, causing reduced efficacy (Humbert *et al*., 1991), although it has been shown that this is not always the case (Bolder and Palmu, 1995).

During the slaughtering process it is difficult to prevent cross-contamination of the carcasses, but the microbiological quality of the end products can be markedly improved by lowering the level of contamination of flocks entering the processing plant. A neck skin sample reflects the hygienic status of the process rather than that of the individual bird. Thus, a considerable decrease in the numbers of *Salmonella*-positive birds and flocks entering the processing plant is needed in order to significantly reduce the number of positive neck skin samples representing process hygiene. The present study showed that the proportion of *Salmonella*-positive neck skin samples were significantly lower when CE-treated birds were processed than during the processing of untreated flocks.

Neck skin samples of the CE-treated flocks were taken after evisceration and the control flocks were sampled after air chilling and wrapping. According to a survey of 12 French poultry processing plants, 8 of which used air chilling, the increase in the proportion of *Salmonella*-positive neck skin samples occurred during scalding and plucking (Salvat *et al*., 1993). The proportion did not increase from evisceration to wrapping, when air chilling was applied. The average contamination rate of neck skin samples in this survey was 50% after evisceration and 52% after air chilling and wrapping.

Comparison between incidences of *Salmonella* in broiler carcasses reported from different countries is difficult because methods of sampling and analyses vary greatly. Carramiñana *et al*. (1994), reported a *Salmonella* incidence of 60% in surface samples of chilled broiler carcasses in Spain. In a survey made in Germany by Fries (1987), 47% of postchill skin samples were *Salmonella*-positive.

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