Mortality and Growth Performance of Broilers Given Drinking Water Supplemented with Chicken-Specific Probiotics

H. M. Timmerman,*‡ A. Veldman,‡ E. van den Elsen,§ F. M. Rombouts,# and A. C. Beynen*1

*Department of Nutrition, Faculty of Veterinary Medicine, Utrecht University, PO Box 80152, 3508 TD Utrecht, The Netherlands; †Research and Development Department, Winclove Bio Industries B.V., PO Box 37239, 1030 AE Amsterdam, The Netherlands; ‡Schothorst Feed Research, PO Box 8200 AM Lelystad, The Netherlands; §Research and Development Department, Fransen mengvoeders B.V., PO Box 30, 5469 ZG Erp, The Netherlands; and #Laboratory of Food Microbiology, Department of Agrotechnology and Food Sciences, Wageningen University, PO Box 8129, 6700 EV Wageningen, The Netherlands

ABSTRACT For application in broiler production, we developed a multispecies (MSPB) and a chicken-specific (CSPB) probiotic preparation in fluid form. The MSPB contained different probiotic species of human origin, whereas the CSPB consisted of 7 Lactobacillus species isolated from the digestive tract of chickens. In a field trial with broilers, MSPB treatment resulted in a slight increase (by 1.84%) in broiler productivity based on an index taking into account daily weight gain, feed efficiency, and mortality. The CSPB treatment reduced mortality in 2 subsequent field trials and raised productivity by 2.94 and 8.70%. In a controlled trial with broilers showing a high index of productivity, probiotic treatment further raised productivity by 3.72%. Based on the present 4 studies in combination with 9 studies published earlier, it is suggested that with higher productivity rates of the broilers the effect of probiotics becomes smaller.

Key words: broiler, multispecies probiotic, chicken-specific probiotic, feed efficiency

INTRODUCTION

Colonization of the digestive tract with commensals induces in the host a set of genes associated with postnatal maturation, mucosal barrier fortification, innate immune responses, and promotion of nutrient metabolism (Hooper et al., 2001; Stappenbeck et al., 2002; Rawls et al., 2004). A well-accepted method to quickly introduce a commensal microflora in hen-deprived chicks is through the administration of probiotics. The most widely used probiotic strains are of the genus Lactobacillus, which is also the dominant genus of the proximal intestine of chickens early in life (Barnes et al., 1972). Edens et al. (1997) showed that in ovo and ex ovo administration of Lactobacillus reuteri resulted in an increased villus height, indicating that probiotics are potentially able to enhance nutrient absorption and thereby improve growth performance and feed efficiency. Indeed, Lactobacillus administration has been shown to improve growth rates and feed (1997) showed that in ovo and ex ovo administration of Lactobacillus reuteri resulted in increased villus height, indicating that probiotics are potentially able to enhance nutrient absorption and thereby improve growth performance and feed efficiency. Indeed, Lactobacillus administration has been shown to improve growth rates and feed (1997) showed that in ovo and ex ovo administration of (Jin et al., 1998a,b, 2000; Zulkifli et al., 2000). However, in challenge experiments with pathogens such as Escherichia coli, Salmonella typhimurium, and Staphylococcus aureus, there was a probiotic-induced reduction in mortality (Watkins and Miller, 1983; Edens et al., 1997).

We have provided evidence that multispecies probiotics (MSPB) are more effective than monospecies probiotics (Timmerman et al., 2004) and also that species-specific probiotics elicit different health effects than do probiotics derived from another host species (Timmerman et al., 2005). To further qualify the potential of probiotics to improve growth performance and mortality in broilers, we investigated the effect of a chicken-specific probiotic (CSPB) that was administered with the drinking water. To evaluate the application of the CSPB in practice, the efficacy was not only studied in a controlled experiment but also in 2 field trials. In an additional field trial we used a MSPB containing different probiotic species of human origin. To perform the 4 experiments, we developed a fermentation medium that is suitable for administration via the drinking water, thereby rendering redundant the use of expensive freeze-dried preparations.

MATERIALS AND METHODS

Probiotic Strains

For this study 2 different liquid probiotic formulas were developed. The MSPB preparation contained commer-
cially available probiotic strains. A combination of 6 strains was used: Lactobacillus acidophilus W55, Lactobacillus salivarius W57, Lactobacillus casei W56, Lactobacillus plantarum W59, Lactococcus lactis W58, and Enterococcus faecium W54 (Winlove Bio Industries B.V., Amsterdam, The Netherlands). To formulate the CSPB preparation, Lactobacillus strains were isolated from fresh digesta and intestinal tissue samples taken from healthy chickens and were screened for probiotic properties.

**Isolation of Lactobacillus Strains from Chickens.**

Digesta and tissue samples of the crop, small intestine, and cecum were collected freshly from layer hens and broiler chickens and were stored in sterile, buffered peptone water (Oxoid, Haarlem, The Netherlands). The samples were kept refrigerated and were processed on the same day. The digesta samples were homogenized in buffered peptone water with an Ultra Turrax blender (Janke and Kunkel, IKA Labortechnik, Staufen, Germany) under anaerobic conditions. Mucosal scrapings were derived from the tissue samples and dissolved in buffered peptone water. Further processing was performed under aerobic conditions. Serial dilutions of the homogenized samples were made in reduced physiological salt solution made as follows (grams per liter): neutralized bacteriological peptone, 1; l-cysteine-HCl, 0.5 (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) and NaCl, 8 (Acros Organics, Geel, Belgium). Lactobacillus strains were cultured on LAMVAB plates selective for Lactobacilli due to low pH (5.0) and the presence of vancomycin in the medium (Hartemink et al., 1997). The plates were incubated anaerobically (Anoxomat, Mart, Lichenvoorde, The Netherlands) at 37°C for 48 h.

Well-isolated colonies with different appearances were picked from each plate and transferred to new LAMVAB plates and further incubated at 37°C for 48 h (anaerobic) to obtain pure strains. Finally, 150 pure colonies were isolated and grown in de Man, Rogosa, and Sharpe broth (Merck, Darmstadt, Germany) at 37°C for 24 h. Aliquots of these cultures were stored with 30% glycerol at −20°C. Well-isolated colonies were picked from each plate and transferred to new LAMVAB plates and further incubated at 37°C for 24 h. Aliquots of these cultures were mixed and a sample was taken to determine the total viable cell count of the finished product (10⁹ cfu/mL). The product was kept refrigerated and was stored for up to 2 wk. During storage the total cell count was checked regularly to verify the stability of the product.

Because of administration problems of the probiotic preparation in field trials 1 and 2 (see later), attempts were made to optimize the probiotic preparation to prevent clogging and sedimentation of probiotic components in the drinking water system in field trial 3. Essentially, the procedure of preparation remained unchanged, except that instead of adding the whole fermentation broth to the drinking water, the probiotic cells were first washed with buffered peptone water by use of crossflow filtration (Sartorius Technologies B.V., Nieuwegein, The Netherlands). During this procedure the final concentration of the product was increased to 1.0 × 10¹⁰ cfu/mL.

**Experimental Design**

**Field Trial 1.** This trial took place at the research station of Fransen Mengvoeders B.V. Five thousand 1-d-old male Cobb broiler chicks (Cobroed, Lievelde, The Netherlands) were housed in a standard broiler house divided in 2 similar areas with separate feed and drinking facilities. Broilers were fed a commercial starter diet from 0 to 14 d of age (ME, 2,850 kcal/kg; CP, 220 g/kg), a grower diet from 14 to 28 d (ME, 3,025 kcal/kg; CP, 202 g/kg), and a finisher diet from d 29 onward (ME, 3,050 kcal/kg; CP, 192 g/kg). The anticoagulant antibiotics nicarbazin (125 mg/kg) and salinomycin (70 mg/kg) were added to the starter and grower diets, respectively. Water and feed were supplied for consumption ad libitum. The MSPB treatment was randomly assigned to one-half of the house and started immediately after arrival of the chicks. Two

**Formulation of the MSPB and CSPB Preparations.**

The liquid growth medium was composed of 200 g of soy protein hydrolysate (Heybroek, Amsterdam, The Netherlands), 160 g of yeast extract (Gistex LS powder AGGL, DSM Food Specialties, Delft, The Netherlands), 200 g of dextrose (Roquette, www.roquette.com), and 80 g of minerals [a combination of potassium chloride, magnesium sulphate, and manganese sulphate (Kortext, The Netherlands)]. These ingredients were dissolved in 15 L of hot tap water, and 25 L of cold tap water was then added. The product was cooled down quickly and stored refrigerated in small containers. For formulation of both the MSPB and CSPB preparation, the component strains (pregrown in de Man, Rogosa, and Sharp broth and stored at −80°C) were first individually inoculated at 10 mL/L and grown overnight at 37°C in the liquid fermentation medium.

After fermentation, the pH of the cultures was determined (pH < 4.2) and the optical density of the cultures was measured using a spectrophotometer (λ = 620 nm) to assure growth of all strains (optical density > 1.00). Also, samples were taken for viable cell count analysis of each strain. Then, equal volumes of each of the 6 cultures were mixed and a sample was taken to determine the total viable cell count of the finished product (10⁹ cfu/mL). The product was kept refrigerated and was stored for up to 2 wk. During storage the total cell count was checked regularly to verify the stability of the product.
times for each strain.

Table 1. Selected Lactobacillus species for the preparation of the liquid chicken-specific probiotic preparation

<table>
<thead>
<tr>
<th>Isolate</th>
<th>pH 5.6</th>
<th>pH 4.0</th>
<th>Acidification</th>
<th>Pathogen inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>Listeria monocytogenes</td>
</tr>
<tr>
<td>W204.5</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>Escherichia coli</td>
</tr>
<tr>
<td>W206.6</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>Salmonella typhimurium</td>
</tr>
<tr>
<td>W218.2</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>Identification</td>
</tr>
<tr>
<td>W223.5</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>W227.3</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>W227.5</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

1Growth rate at different pH levels was assessed as short lag phase, followed by rapid growth in the exponential phase (steep slope) and a high total cell count at the end of the experiment (measured by optical density), designated as (+ +). Strains with (+) showed a lower optical density value at the end of the experiment. The experiment was performed 3 times for each strain.

2Acidification was assessed as a change in color (at \( \gamma = 405 \) nm in the microplate reader) during 32 h of the pH-indicators bromo-cresol green and purple. A fast and high increase is indicated with ++, a slower or less intensive increase is indicated with +. The experiment was performed 3 times for each strain.

3Inhibition is measured as the diameter (mm) of the inhibition zone for all pathogens. The values in the table are the average of 2 measurements.

4Identification according to the carbohydrate fermentation profile (API 50 CH, BioMérieux, Inc., Hazelwood, Mo).

Table 2. Growth performance and mortality of broilers in 3 pooled field trials and a controlled trial with administration of a multispecies probiotic (MSPB) or a chicken-specific probiotic (CSPB) in the drinking water

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Pooled data field trials1</th>
<th>Controlled trial2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Probiotic</td>
</tr>
<tr>
<td>Final BW, g</td>
<td>2,357</td>
<td>2,342</td>
</tr>
<tr>
<td>Feed intake, g</td>
<td>4,460</td>
<td>4,300</td>
</tr>
<tr>
<td>Average daily gain, g/day</td>
<td>49.99</td>
<td>49.65</td>
</tr>
<tr>
<td>Feed conversion, kg of feed/kg of gain</td>
<td>1.93</td>
<td>1.87</td>
</tr>
<tr>
<td>Flock total BW, kg</td>
<td>5,448</td>
<td>5,506</td>
</tr>
<tr>
<td>Mortality rate, %</td>
<td>8.84</td>
<td>7.27</td>
</tr>
</tbody>
</table>

1Data are means of 3 field trials. In field trial 1 the MSPB was applied. In field trials 2 and 3 the CSPB was applied.

2Data are means of 6 replicate pens of 35 birds each.

Data Collection

In field trials 1–3, BW was recorded only at the start and at the end of the experiment. Body weight at d 0 was assessed as the average of a random selection of 250 chicks per experimental flock. Final BW was assessed by dividing the total weight per experimental flock by the number of chicks alive before transportation to the processing plant. Feed and water consumption on a flock basis was recorded daily. In the controlled trial, weight gain and feed intake were recorded for 3 growth stages (starter: d 0 to 14; grower d 14 to 30; and finisher: d 30 to 39). In all trials mortality was recorded daily, and percentage divided into 12 groups of 35 chicks each. Each group was assigned to a floor pen that contained a self-feeder and waterer to provide ad libitum access to feed and water. Broilers were fed a commercial starter diet from 0 to 14 d of age (ME, 2,775 kcal/kg; CP, 205 g/kg), a grower diet from 14 to 30 d, and a similar finisher diet from 31 to 37 d (ME, 2,900 kcal/kg; CP, 200 g/kg). The anticoccidial antibiotics diclazuril (1 mg/kg) and monensin (100 mg/kg) were added to the starter and grower diets, respectively. Probiotic treatment was randomly assigned to 6 groups of 35 chicks. Spraying and supplementation rates of CSPB were identical to those described for field trial 2.
mortality was calculated. Feed conversion was calculated per experimental unit as total feed intake (kg): total gain of live chickens (kg).

**Statistical Analysis**

The data for each variable were subjected to 1-way ANOVA (Steel and Torrie, 1980). Differences between treatment groups were evaluated with the Student’s t-test using the GLM procedure of SAS (SAS Institute, 2000). The level of statistical significance was preset at $P < 0.05$ for 1-sided testing. Based on literature (Watkins and Kratzer, 1984; Jin et al., 1998a,b, 2000; Abdulrahim et al., 1999; Kalavathy et al., 2003) it was expected that the treatment effect goes in 1 direction. Mortality within experiments was evaluated by means of Fisher’s exact test.

**RESULTS**

Growth performance and mortality for all field trials are pooled in Table 2. Figure 1 shows the time course of cumulative mortality. In field trial 1, administration of the MSPB preparation had no effect on weight gain and feed conversion (data not shown). Overall mortality was similar for the control and MSPB-treated groups, but Figure 1 illustrates that until 42 d the mortality was lower in the MSPB group. During the last week of the experiment ambient temperature was extremely high, which probably caused the rapid increase in mortality in both groups. The increase was greater in the broilers given MSPB so that overall mortality in the 2 groups became similar. In field trial 2 the newly developed CSPB was used. The initial progress of the trial was complicated by an *E. coli* infection of the yolk sac, which became evident on d 2. There was a sharp rise in initial mortality. When compared with the control birds, the probiotic-treated chicks, which were kept in the same house, were visually less affected by the infection. During the first 3 d, 27 chicks in the probiotic-treated group died compared with 73 in the control group. To prevent excessive mortality, all animals were treated with a combination of sulfamethoxazole (80%) and trimethoprim [20%; T.S. SOL (Dopharma, www.dopharma.com)] from d 3 to 6. During antibiotic treatment, probiotic treatment was stopped because in-
vitro experiments had shown that the CSPB strains were highly sensitive to the antibiotic used. After antibiotic treatment, probiotic treatment was reinstalled. The CSPB treatment caused a slight decrease in overall mortality and also produced an improvement of feed conversion (data not shown). In field trials 1 and 2 it was observed frequently that insoluble fermentation metabolites sedimented and subsequently clogged the dosage pump. In the controlled trial, the results of which are given later, the chicks given drinking water with CSPB drank less. Possibly, this was related to an adverse taste response of the water due to the organic acids present in the CSPB preparation. Neutralization of the acids with calcium carbonate raised water intake. It was decided to separate the medium and the probiotic cells by washing the cells with buffered peptone water as described in the Materials and Methods section. The improved CSPB preparation was used in field trial 3, and the technical problems seen in the earlier experiment were prevented. Probiotic treatment resulted in a clear improvement of the feed conversion (data not shown) and a decrease in mortality.

The design of the field trials was such that in each trial there was only 1 experimental unit per treatment. To carry out statistical analysis, the data of the 3 field trials were pooled even though different probiotic preparations had been used. After pooling of the data, probiotic treatment appeared to have caused a statistically significant improvement of feed conversion. The probiotic-induced decrease in overall mortality just failed to reach statistical significance.

A controlled trial was carried out with the probiotic preparation used in field trial 2. The results are presented in Table 2. The treatment with CSPB decreased feed conversion in the starting period (d 0 to 14; data not shown). Average daily gain during the growth phase (14 to 30 d) and BW at 30 d were significantly higher in the CSPB-treated group (data not shown). This growth-promoting effect was not present at the end of the trial. The CSPB-mediated reduction in mortality was similar to that seen for field trial 3, but it was not statistically significant (Figure 1; Table 2).

**DISCUSSION**

It is clear from our studies that the administration of probiotics via the drinking water had beneficial effects on broiler performance. In the field trials, probiotic treatment significantly improved feed conversion. In each field trial total final BW was increased by supplemental probiotics, ranging from 0.74 to 1.64%. Mortality was reduced by the addition of probiotics to the drinking water. In the controlled trial there was a marked decrease in mortality after probiotic administration, which was associated with a 4.87% increase in total final BW. Unlike in the field trials, feed conversion was not influenced in the controlled trial, which may relate to the favorable feed conversion rates in the latter trial. When the data for total final BW and mortality were pooled for the 4 experiments, the percentage increase in weight and the percentage decrease in mortality were found to be at least borderline statistically significant (P = 0.065 and P = 0.038). An index of productivity is the so-called production number, which equals kilograms of growth per day * (100% – mortality) / Feed conversion * 100 (Voeten, 1974). Production numbers could not be calculated for all studies applying Lactobacillus preparations because mortality was not always mentioned. Details as to reference, number of animals per treatment, breed, probiotic description, daily dosage, and administration route are as follows: 1, Jin et al. (1998a), 6 cages * 10 chicks, Arbor Acres, Lactobacillus acidophilus (1a) or a mixture of 12 Lactobacillus strains (1b), 1-2 × 10^6 cfu/g diet, feed; 2, Jin et al. (1998b), 10 cages * 50 chicks, Arbor Acres, Lactobacillus casei, 0.5-1.0 × 10^6 cfu/g diet (2a) or 1-2 × 10^6 cfu/g diet (2b) or 2-4 × 10^6 cfu/g diet (2c), feed; 3, Jin et al. (2000), 5 cages * 12 chicks, Arbor Acres, Lactobacillus acidophilus (3a) or a mixture of 12 Lactobacillus strains (3b), 1-2 × 10^6 cfu/g diet, feed; 4, Watkins and Kratzer (1984), 2 cages * 50 chicks, not mentioned, undefined Lactobacillus culture, 2 × 10^6 cfu/chick, water; 5, Zulkifli et al. (2000), 12 cages * 10 chicks, Shaver × Shaver and Hubbard × Hubbard, mixture of 12 Lactobacillus strains, 1-2 × 10^6 cfu/g of diet, feed.

In a study with veal calves we also noted that growth performance of the control group was negatively associated with the magnitude of the effect of probiotics (Timmerman et al., 2005). Differences in the administration of probiotics might be another factor affecting efficacy. Administration of probiotics in the drinking water (Watkins and Kratzer, 1984; present trials) generally resulted in a lower increase of average daily gain when compared with studies with probiotic administration via the feed (Yeo and Kim, 1997; Jin et al., 1998a,b, 2000; Abdulrahim et al., 1999; Zulkifli et al., 2000; Kalavathy et al., 2003). Another determinant of probiotic efficacy may be the timing of administration. During early life, colonization patterns are instable and chicks are then susceptible to environmental pathogens. Initial colonization is of great importance to the host because the bacteria can modulate expression of genes in epithelial cells (Hooper et al., 2001), thus creating a favorable habitat for themselves. These pioneering bacteria will
probably reside in the intestine permanently and determine the colonisation pattern of bacteria introduced later in life (Ducluzeau, 1993). The primary colonizers are therefore relevant to the final composition of the permanent flora in full-grown chickens. Prebiotic compounds supplied early in life may prove beneficial in aiding permanent colonization of the probiotic strains administered, beneficial indigenous microbes, or both.

Overall, probiotic treatment induced a clear reduction in mortality. Further research is required to study underlying mechanisms and to evaluate the economic impact of the use of probiotics in broilers. Further experiments with the improved CSPB, as applied in field trial 3, are required to test whether this less expensive probiotic is as effective as freeze-dried preparations, which can be applied via the feed.

ACKNOWLEDGMENTS

The research was supported in part by the Dutch Ministry of Economic Affairs (SENTER). The authors wish to thank R. Blankenstein, L. Mulder, and C. Warmerdam for technical assistance.

REFERENCES


