Effect of BROILACT® on the Physicochemical Conditions and Nutrient Digestibility in the Gastrointestinal Tract of Broilers

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ABSTRACT The effect of the competitive exclusion (CE) product BROILACT® on Salmonella colonization, nutrient digestibility, and the ME of the feed and the production of volatile fatty acids in the chicken gut was evaluated. The ileal viscosity and the fecal dry matter content were also determined. Newly hatched broiler chicks were given BROILACT® orally either once on the day of hatch or five times during a period of 2 wk. Samples were taken at 12 and 31 d of age. In the beginning of the study and 2 wk later, chicks from each treatment group were taken to separate facilities to be challenged with Salmonella. Five and 4 d later, the chicks were killed and their intestines were examined for Salmonella. The results of the present study show that BROILACT® protected the chicks against Salmonella, decreased the viscosity of the ileal contents, and increased the fecal dry matter content, and increased the ME value of the feed by 1.6% and the concentration of propionic acid in the cecal contents.

(Key words: competitive exclusion, poultry production, chicken nutrition, normal gut microflora)

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INTRODUCTION

Competitive exclusion (CE) or the Nurmi Concept together with conventional hygienic measures, is a well known and thoroughly investigated measure against food-poisoning Salmonella in poultry (Nurmi and Rantala, 1973; Pivnick and Nurmi, 1982; Mead and Impyey, 1987; Schneitz, 1993).

The concept was originally devised to control Salmonella infections, but it has been shown experimentally that CE treatment also protects chicks against pathogenic Escherichia coli (Hakkinen and Schneitz, 1996), Yersinia (Soerjadi-Liem et al., 1984), and Campylobacter spp. (Aho et al., 1992; Stern, 1994; Mead et al., 1996). It has also been shown that CE treatment decreases mortality due to necrotic enteritis and reduces the counts of Clostridium perfringens, which is one of the causative factors in necrotic enteritis (Elwinger et al., 1992).

Claims have also been made that CE treatment enhances the growth and decreases the mortality of birds. According to Goren et al. (1984), an improvement in growth rate was observed in commercial broiler flocks sprayed with an undefined CE culture. Corrier et al. (1995a) reported an improvement in the efficiency of feed utilization in broiler flocks that were given CE treatment on the day of hatch. They also noticed a significant increase in the concentration of propionic acid in the cecal contents of the CE-treated chicks. The increase was similar to that reported previously by Corrier et al. (1995b) in laboratory trials. An improvement in bird performance in terms of higher body weight, feed consumption, and feed conversion, in addition to lower mortality, was obtained by Abu-Ruwaida et al. (1995). Higher body weight and lower mortality were also noticed by Bolder et al. (1995) in CE-treated flocks. No explanation for the improvement of broiler performance by CE has been expressed.

BROILACT® is the first commercial CE product and was developed by Orion Corporation in Finland. It was launched in Finland and Sweden in 1987 and, until 1994, it was sold in liquid form. After that, the lyophilized product substituted the original preparation. BROILACT® is a well characterized mixture of selected chicken intestinal bacteria.

This study was undertaken to evaluate the effect of single or multiple doses of BROILACT® on the nutrient digestibility, the ME, and the production of volatile fatty
acids (VFA) in the chicken gut. On the day of hatch and at 2 wk of age, chicks from each experimental group were taken to separate rearing facilities to be challenged with a high dose of *Salmonella*. This was done to test the efficacy of BROILACT® when given either once on the day of hatch or five times over a period of 2 wk.

**MATERIALS AND METHODS**

**Test Material**

BROILACT® is a strongly selected mixture of bacteria derived from the cecal contents of a healthy adult hen (Nurmi et al., 1987). The composition consists of 32 identified bacteria able to adhere to the gut wall of the bird. Clostridia and other poultry and human pathogens have been excluded from the product.

**Test Animals**

Commercial Ross × Ross broiler chicks were brought from a commercial hatchery. The chicks were sexed and 360 male chicks divided into three experimental groups were used in this study.

**Feed and Water**

A commercial starter diet without growth-promoting antibiotics was consumed *ad libitum*. The feed contained soybean meal, 28%; wheat, 15%; barley, 25%; and dehulled oats, 20%. Narasin (70 mg/kg) was used as coccidiostat and the feed was supplemented with phytase (Natuphos) and β-glucanase and xylanase (Econase wheat). The crude protein content of the feed was 22.0% and the ME value was 2,386 kcal/kg.

When the chicks were about 2.5 wk of age, the same feed was mixed after milling with 1% of silicate (Celite 545) and pelleted using a 4-mm matrix. This method was used to determine acid insoluble ash (insoluble in 4N HCl) using the AIA method (Vogtmann et al., 1975; van Keulen and Young, 1977). This feed was used until the end of the trial.

Regular tap water was consumed *ad libitum* either from nipple drinkers or from a trough (chicken assay test).

**Challenge Organism**

The challenge organism was a nalidixic acid resistant derivate of *Salmonella infantis* originally isolated from a broiler chicken.

**Media and Antisera**

Brain heart infusion broth containing 25 mg/L of nalidixic acid was used for prechallenge cultivation of the challenge organism. Bromthymolblue-lactose-sucrose agar containing 25 mg/L of nalidixic acid was used to selective isolation and enumeration of the challenge organism. Rappaport Vassiliadis was used for sample enrichment of *Salmonella* and *Salmonella* 07-antiserum was used for the agglutination test.

**Experimental Design**

The study consisted of three treatment groups: 1) untreated control group 2) 1 mg of BROILACT® in a dose volume of 0.3 mL given once on the day of hatch; 3) 1 mg of BROILACT® in a dose volume of 0.3 mL given five times with an interval of from 2 to 5 d during a period of 2 wk starting the 1st d after hatch. BROILACT® was given to the chicks orally by gavage.

In the beginning of the study, each group consisted of 120 Ross male chicks. Birds in Groups 2 and 3 were randomly placed in cages located at both ends of a three-tier battery (12 cages per group with 10 chicks in each cage). The size of the cages was 48 × 56 cm. The two treatment groups were separated with four empty cages, i.e., nearly 2 m. The chicks in Group 1 were randomly placed in cages in a one-tier battery at a distance of 2 m from the three-tier battery and separated from that by a plastic curtain (six cages with 20 chicks in each cage). The size of these cages was 96 × 56 cm.

**Chicken Assay Trials**

**Trial 1.** In the beginning of the trial, 20 BROILACT® treated and 30 untreated control chicks were taken to the rearing facilities at Turku University, divided into groups of 10 and placed in cardboard boxes. The BROILACT® treated chicks and 2 × 10 untreated control chicks were challenged the following day orally by gavage with 5.0 × 10^4 viable *Salmonella infantis* cells per bird. One group with 10 chicks was left as negative control. The chicks were asphyxiated with carbon dioxide 5 d later and their intestines were examined for *Salmonella*.

**Trial 2.** The procedure was repeated when the chicks were 14 d old. Altogether, 54 chicks were randomly taken from each treatment group and divided in groups of 6 chicks: 3 × 6 untreated control chicks, 3 × 6 from Group 2, and 3 × 6 from Group 3. The following day, 2 × 6 untreated control chicks and all BROILACT®-treated chicks were challenged orally by gavage with 6.0 × 10^7 viable S. infantis cells per bird. One 1 × 6 group of chicks was left as a negative control. The chicks were asphyxiated with carbon dioxide 4 d later and their intestines were examined for *Salmonella*.

**Sampling**

At the ages of 12 and 31 d, four chicks were killed by cervical dislocation from each of the large cages (Group 1)
and 2 from each of the small cages (Groups 2 and 3) to collect intestinal contents from duodenum, ileum, and ceca. The intestinal contents of the four chicks from each of the large cages were pooled and likewise those from two small cages, resulting in six samples per treatment group.

At the age of 24 to 26 d, uncontaminated feces was collected from the plates or plastic plates covered under the cages. The fecal samples from two small cages next to each other were pooled. Thus, the digestibility test also included six replicates per treatment group. After the fecal collection, two chicks were taken from each large cage and one chick was taken from each small cage. The chicks were euthanatized and the ileal viscosity was determined using the Brookfield digital viscosimeter DV-II (H 5308).^{10}

The digesta pH was measured for the different segments of the intestine and from the ileal and cecal contents the concentrations of VFA were also determined. From the samples collected at the age of 31 d the concentration of lactic acid was also determined. Before measuring the pH the sample was diluted 1:1 with ionized water. The ileal and cecal samples were diluted 1:10 before determining the lactic acid and VFA concentrations. The samples were extracted and from the filtered extract the determination of lactic acid was performed colorimetrically (Barker and Summerton, 1941; Haacker et al., 1983).

For determination of VFA, two drops of mercuric chloride and 1.25 mL of formic acid were pipetted into a 25-mL measuring bottle and filled with the extract. The determination was done using a HP 5890 gas chromatograph with an automatic injector HP 7673, F 10 detector and HP 3365 series II chemstation (column HP-FFAP 10 m × 0.53 × 1.0 mm, carrier gas helium).^{11} In the determination of the results an external standardization was used.

For the feed and feces used in the determination of the digestibility, the dry matter content, total nitrogen, ash (AOAC, 1984), and the gross energy (GE) were determined. An IKA calorimeter C 400^{12} was used to determine the GE value. The same parameters with the exception of GE were determined from the ileal contents. The AIA was used as an indicator in the determination of digestibilities, ME, and retention of nitrogen (Vogtmann et al., 1975; van Keulen and Young, 1977).

### Calculations

Apparent digestibilities and ME of the diet were calculated using the following formulas:

\[
\text{Digestibility or retention percentage} = 100 - \left[100 \times \left(\frac{\text{Dietary AIA cont.}}{\text{Fecal and ileal AIA cont.}} \times \frac{\text{Fecal and ileal nutrient cont.}}{\text{Dietary nutrient cont.}}\right)\right] \\
\text{ME} = \text{GE of the feed} - \left(\text{GE of the feces} \times \frac{\text{AIA of the feed}}{\text{AIA of the feces}}\right)
\]

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### RESULTS AND DISCUSSION

The results of this study are presented in Tables 1 to 4. Table 1 shows the results of the chicken assay test. The chicks treated with BROILACT® either once on Day 1 or five times during a period of 14 d were well-protected against a high dose of *Salmonella*. There were no differences in the efficacy against *Salmonella* between the two BROILACT® treatments. The results also show that 2-wk-old chicks are already difficult to challenge even with a high dose of the challenge organism because of the development of the intestinal microflora.

The pH and VFA and lactic acid concentrations in different sections of the gut are shown in Tables 2 (samples taken at 12 d of age) and 3 (samples taken at 31 d of age). Because the site of bacterial fermentation in birds is mainly the ceca (Annison et al., 1968), the results are logical because in each case the concentrations of VFA in the cecal contents were higher than in the ileal contents. The concentration of lactic acid showed an inverse pattern. The concentration of acetic acid in the ileum and ceca was clearly higher than the concentration of the other acids.

At 12 d of age, the concentration of butyric acid was significantly lower (P < 0.05) in the ileal contents of the BROILACT®-treated chicks, which may reflect the fact that BROILACT® does not contain any clostridia and has been shown to decrease the incidence of necrotic enteritis in broiler chickens and decrease the counts of *Clostridium perfringens* in the chicken ceca (Elwinger et al., 1992). Although the picture of necrotic enteritis is not fully clear, it is likely that when changes in intestinal
TABLE 2. Effects of the BROILACT® treatments on pH and volatile fatty acid concentrations (VFA) in the intestines of the broiler chickens at 12 d of age

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>BROILACT® 1 ×</th>
<th>BROILACT® 5 ×</th>
<th>SEM</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH duodenum</td>
<td>6.02</td>
<td>5.97</td>
<td>5.97</td>
<td>0.011</td>
<td>NS</td>
</tr>
<tr>
<td>pH ileum</td>
<td>7.45</td>
<td>7.75</td>
<td>7.56</td>
<td>0.066</td>
<td>NS</td>
</tr>
<tr>
<td>pH ceca</td>
<td>5.24</td>
<td>5.32</td>
<td>5.63</td>
<td>0.081</td>
<td>NS</td>
</tr>
<tr>
<td>Ileum, μmol/g</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetic acid</td>
<td>46.60</td>
<td>55.00</td>
<td>31.60</td>
<td>4.00</td>
<td>NS (P = 0.054)</td>
</tr>
<tr>
<td>Propionic</td>
<td>0.36</td>
<td>0.45</td>
<td>0.43</td>
<td>0.018</td>
<td>NS (P = 0.075)</td>
</tr>
<tr>
<td>Butyric</td>
<td>0.20ab</td>
<td>0.06ab</td>
<td>tracesb</td>
<td>0.033</td>
<td>*</td>
</tr>
<tr>
<td>Isovaleric</td>
<td>0.22</td>
<td>0.17</td>
<td>0.15</td>
<td>0.013</td>
<td>NS (P = 0.08)</td>
</tr>
<tr>
<td>Caprylic</td>
<td>0.03b</td>
<td>0.15a</td>
<td>0.15a</td>
<td>0.021</td>
<td>**</td>
</tr>
<tr>
<td>Ceca, μmol/g</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetic acid</td>
<td>109.90</td>
<td>109.90</td>
<td>118.20</td>
<td>3.830</td>
<td>NS</td>
</tr>
<tr>
<td>Propionic</td>
<td>6.80b</td>
<td>16.20ab</td>
<td>17.50a</td>
<td>1.890</td>
<td>*</td>
</tr>
<tr>
<td>Isobutyrinic</td>
<td>0.31</td>
<td>0.51</td>
<td>0.62</td>
<td>0.057</td>
<td>NS (P = 0.055)</td>
</tr>
<tr>
<td>Butyric</td>
<td>14.80</td>
<td>13.60</td>
<td>18.20</td>
<td>1.130</td>
<td>NS</td>
</tr>
<tr>
<td>Isovaleric</td>
<td>0.29b</td>
<td>0.59ab</td>
<td>0.69a</td>
<td>0.059</td>
<td>*</td>
</tr>
<tr>
<td>Valeric</td>
<td>0.59b</td>
<td>1.27ab</td>
<td>1.57a</td>
<td>0.166</td>
<td>*</td>
</tr>
</tbody>
</table>

a,bMeans within a row with no common superscript differ significantly (P < 0.05).

*p < 0.05.

**p < 0.01.

environment take place, the number of C. perfringens may increase, which, in turn, results in a greater production of alpha-toxin by C. perfringens, and outbreak of necrotic enteritis may be induced (Fukata et al., 1991). Sakata (1987) demonstrated in rats that intraluminally infused VFA accelerated the crypt cell production rate and increased the gut-wall mass. The stimulation was most efficient with butyrate. The positive effect of dietary antibacterials appears to be related to the elimination of fermentative microorganisms, mainly butyric acid producers (especially clostridia), from the small intestine (Choc et al., 1996). This effect has been shown to decrease the gut-wall mass and stimulate nutrient absorption (Parker and Armstrong, 1987; Visek, 1978), and supports the improved nutrient digestibility found in the present study.

BROILACT® increased slightly the production of propionic acid and clearly that of capronic acid (P < 0.01) in the ileum at 12 d of age. The cecal samples taken at the same age showed an increase in the concentration of propionic (P < 0.05), isovaleric, and valeric acids.

TABLE 3. Effects of the BROILACT® treatments on pH, volatile fatty acids (VFA) and lactic acid concentrations in the intestines of the broiler chickens at 31 d of age

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>BROILACT® 1 ×</th>
<th>BROILACT® 5 ×</th>
<th>SEM</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH duodenum</td>
<td>5.94</td>
<td>5.95</td>
<td>6.05</td>
<td>0.024</td>
<td>NS</td>
</tr>
<tr>
<td>pH ileum</td>
<td>7.45</td>
<td>7.63</td>
<td>7.76</td>
<td>0.061</td>
<td>NS</td>
</tr>
<tr>
<td>pH ceca</td>
<td>5.99</td>
<td>5.89</td>
<td>5.94</td>
<td>0.052</td>
<td>NS</td>
</tr>
<tr>
<td>Ileum, μmol/g</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetic acid</td>
<td>46.60</td>
<td>51.60</td>
<td>53.30</td>
<td>1.000</td>
<td>NS</td>
</tr>
<tr>
<td>Propionic</td>
<td>traces</td>
<td>traces</td>
<td>traces</td>
<td>. . .</td>
<td>. . .</td>
</tr>
<tr>
<td>Butyric</td>
<td>traces</td>
<td>traces</td>
<td>traces</td>
<td>. . .</td>
<td>. . .</td>
</tr>
<tr>
<td>Lactic</td>
<td>18.90</td>
<td>5.60</td>
<td>8.90</td>
<td>3.330</td>
<td>NS</td>
</tr>
<tr>
<td>Ceca, μmol/g</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetic acid</td>
<td>146.50</td>
<td>133.20</td>
<td>131.60</td>
<td>2.830</td>
<td>*</td>
</tr>
<tr>
<td>Propionic</td>
<td>8.10b</td>
<td>21.60a</td>
<td>22.90a</td>
<td>1.750</td>
<td>***</td>
</tr>
<tr>
<td>Isobutyrinic</td>
<td>0.83</td>
<td>0.99</td>
<td>0.91</td>
<td>0.047</td>
<td>NS</td>
</tr>
<tr>
<td>Butyric</td>
<td>26.10</td>
<td>21.60</td>
<td>20.40</td>
<td>1.020</td>
<td>NS (P = 0.09)</td>
</tr>
<tr>
<td>Isovaleric</td>
<td>0.98</td>
<td>0.98</td>
<td>0.78</td>
<td>0.137</td>
<td>NS</td>
</tr>
<tr>
<td>Valeric</td>
<td>1.80</td>
<td>2.50</td>
<td>2.60</td>
<td>0.140</td>
<td>NS (P = 0.051)</td>
</tr>
<tr>
<td>Capronic</td>
<td>0.20</td>
<td>0.03</td>
<td>0.04</td>
<td>0.050</td>
<td>NS</td>
</tr>
<tr>
<td>Lactic</td>
<td>2.00a</td>
<td>1.40b</td>
<td>1.30a</td>
<td>0.100</td>
<td>**</td>
</tr>
</tbody>
</table>

a,bMeans within a row with no common superscript differ significantly (P < 0.05).

*p < 0.05.

**p < 0.01.

***p < 0.001.
The second determination that was done at 31 d of age showed that BROILACT® slightly increased the production of acetic acid but decreased that of lactic acid in the ileum. In the cecal contents, the concentrations of acetic (P < 0.05), butyric (P < 0.1), and capronic and lactic acids (P < 0.01) decreased. On the other hand, the concentration of propionic acid increased (P < 0.001). This result is in accordance with the results reported by other research groups (Corrier et al., 1995a,b) and the fact that the majority of the anaerobes in BROILACT® produce high amounts of propionic acid. The decrease in the concentration of lactic acid both in the ileal and cecal contents also proves the presence of bacteria that are able to turn lactate to propionate. The increase in valeric acid concentration was not significant (P > 0.05), but the effect of the two treatments was the same. The effects of the two BROILACT® treatments were equal. There were no significant differences in the pH between the groups. The pH of the duodenum was on the average 6.0, that of the ileum 7.6, and that of the ceca at 12 d of age 5.4 and at 31 d of age 5.9.

Both BROILACT® treatments decreased the viscosity of the ileal contents significantly (P < 0.001) (Table 4). Both BROILACT® treatments increased the fecal dry matter content significantly (P < 0.05), but the two treatments did not differ from each other.

Intestinal viscosity is known to be a major factor limiting bird performance (Bedford and Morgan, 1996). Increasing viscosity reduces mixing and feed passage rate (van der Klis et al., 1993). Soluble arabinoxylans in rye and wheat and β-glucans in barley are known to give rise to highly viscous conditions in the small intestine of chicks (Hesselman and Aman, 1986; Chocf and Annison, 1992; Brenes et al., 1993; Bedford and Morgan, 1996; Chocf et al., 1996). To overcome these effects, exogenous enzyme preparations are added to poultry diets. The use of enzymes is very common in many countries where the predominant cereals are wheat and barley. Despite the fact that the ways in which enzymes function are not really understood, they are well accepted by the industry (Bedford and Morgan, 1996). In Finland, enzymes are routinely added to the poultry feed.

The BROILACT® treatment improved the ME value of the feed from 3,220 kcal to 3,270 to 3,273 kcal/kg dry matter (Table 4). The increase was slight (1.6%) but significant (P < 0.001). This result is well in accordance with the positive results gained from the field (Bolder et al., 1995). On the other hand, the effect of BROILACT® on feed utilization may even be more extensive in the field than under laboratory conditions. The digestibility of organic matter increased by 1% (P < 0.001). The nitrogen retention also increased by 1.5 to 3.0% and in the birds given BROILACT® five times the increase was significant (P < 0.05). Ileal digestibility was also improved by 1.5 to 2.0% but the difference was not significant. By decreasing the viscosity of the digesta in the small intestine, BROILACT® improved the nutrient digestibility, which resulted in a higher ME value.

There has been speculation on the role of the bacterial population in the intestine. There is evidence that certain types of diets predispose the birds to certain diseases, e.g., wheat and barley to necrotic enteritis (Elwinger et al., 1992; Kaldhusdal and Hofshagen, 1992). The mechanism for such relationships is not known but may be linked to the viscosity of the intestinal contents (Bedford, 1996). Chocf et al. (1996) demonstrated that increased fermentation occurs in the small intestine when a large amount of viscous nonstarch polysaccharides is present in the diet, and this is detrimental to the performance and well-being of the bird. Chocf et al. (1996) also showed that by enzyme supplementation the ileal fermentation was inhibited, whereas the cecal fatty acid concentration was markedly increased.

Most of the predominant anaerobes in the chicken cecum are able to ferment glucose, which reflects their
mainly saccharolytic nature (Mead, 1989). The occurrence of VFA was already demonstrated in 1960, and it was subsequently reported that strains of *Bacteroides* in the ceca fermented glucose with the production of acetic and propionic acids (Annison et al., 1968). Lactose is of very low energy value for chickens because the birds lack sufficient lactase activity, allowing lactose to enter the ceca. Ability to ferment lactose is also a common property of the major bacterial groups harbored in the chicken ceca. Some cecal anaerobes have been reported to be capable of hydrolyzing starch and some to grow on arabinoxylan (Mead, 1989). Some microbes, e.g., lactobacilli, may also be capable of producing β-glucanases (Bedford, 1996; Chocot et al., 1996).

According to the results of this study, the selected bacterial population of BROILACT®, which is able to attach to epithelial cells and protect the bird against *Salmonella* colonization, improved the degradation of β-glucans and arabinoxylans by increasing the enzymatic activity from that already in the feed, resulting in lower viscosity of the ileal contents, a higher ME value of the feed and increased propionic acid concentration of the cecal contents.

REFERENCES


prophylaxis of intestinal disturbances in poultry. United States patent 4,689,226.


