Preventive Effect of Partially Hydrolyzed Guar Gum on Infection of *Salmonella enteritidis* in Young and Laying Hens

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ABSTRACT

The preventive effect of partially hydrolyzed guar gum (PHGG) on the colonization of *Salmonella enteritidis* (SE) in young and laying hens was investigated. The effects of feed supplemented with 0.025, 0.05, and 0.1% PHGG was examined on young hens orally infected with SE. The incidence of SE in organs was decreased, the excretion of SE into feces was increased, and the agglutinating antibody titer to SE in serum was decreased by the administration of PHGG to young hens. In particular, feed supplemented with 0.025% PHGG was the most effective. It was also shown that feed supplemented with 0.025% PHGG increased the number of *Bifidobacterium* spp. and *Lactobacillus* spp., the most numerous intestinal bacteria in the cecum of young hen. The effect of the excretion of SE via feces was also observed in an experiment using laying hens. The incidence of SE on the surface of the eggshell and in egg white and egg yolk was also decreased when the feed of laying hens was supplemented with 0.025% PHGG. These results show that the administration of feed supplemented with PHGG can prevent the colonization of SE in young and laying hens, which, in turn, could be related to improvement in the balance of intestinal microflora.

(Key words: partially hydrolyzed guar gum, *Salmonella enteritidis*, young hens, laying hens, infection)

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INTRODUCTION

Hen eggs, which are highly nutritious, are consumed worldwide. Because many people eat eggs almost daily, egg contamination is a concern. Recently, serious contamination by *Salmonella* spp. in and on eggs has been reported in Europe, the US (Center for Disease Control, 1996; Wilson et al., 1998), and Japan (Konuma et al., 1995). The infection of *Salmonella enteritidis* (SE) in poultry is one of the most serious problems in the poultry industry because of related outbreaks of food poisoning (Hennessy et al., 1996; Dodhia et al., 1998; Vought and Tatini, 1998).

Much effort has been made to inhibit *Salmonella* spp. infection in poultry. The competitive exclusion method developed by Nurmi and Rantala (Nurmi and Rantala, 1973) is effective in protecting chicks against the colonization of *Salmonella* spp. (Wierup et al., 1988, 1992; Hirn et al., 1992; Nurmi et al., 1992). However, the colonization of *Salmonella* spp. cannot be prevented by this method when breeding farms are contaminated (Nurmi et al., 1992; Nakamura et al., 1995). The inhibition of *Salmonella* spp. with antibiotics does not seem to be practical because of the occurrence of resistant bacterial strains and the residual toxicity of antibiotics in poultry (Boonmar et al., 1998; Gross et al., 1998). Recently vaccination has become common, but has a number of limitations. Vaccine therapy cannot distinguish whether the origin of the antibody derives from the vaccine or a bacterial pathogen when *Salmonella* serology is done. Thus, the period of vaccination is restricted or strictly programmed (Jensen, 1994).

It has been reported that some kinds of carbohydrates, yeast, and, especially, the mannose residue are effective in preventing the *Salmonella* spp. colonization (Oyofo et al., 1989b; Line et al., 1997, 1998). Partially hydrolyzed guar gum (PHGG) was prepared by selectively cleaving the mannan backbone-chain of guar gum by using endo-β-D-mannanase and is composed of galactomannan with a molecular weight of about 20,000 Da (McCleary, 1979a,b; Balascio et al., 1981; Stephen, 1983). Guar gum is produced from the seeds of the guar bean, *Cyamoposis tetragonolobus* L. The PHGG has been reported to reduce diarrhea (Takahashi et al., 1993) and constipation (Takahashi et al., 1994a) in humans, increase the bioavailability of dietary iron (Takahashi et al., 1994c), improve lipid metabolism (Ide et al., 1991), and lower blood glucose level in a human volunteer study (Tsuda et al., 1998). The PHGG has also been shown to improve intestinal

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Abbreviation Key: DHL = deoxycholate hydrogen sulfate lactose; HTT = Hajna tetrathionate; PHGG = partially hydrolyzed guar gum; SE = *Salmonella enteritidis*; TS = tryptic soy.
microflora in vivo in humans (Okubo et al., 1994) and animals (Takahashi et al., 1995).

The present study was conducted to investigate the in vivo inhibitory effect of feed supplemented with PHGG on the colonization of SE in pullets and laying hens after oral injection of SE.

**MATERIALS AND METHODS**

**Feed Preparation**

Teapesc® G300 was prepared for poultry feed mix and comprised 98.2% PHGG, 3.0% water, 1.1% ash, and 0.7% protein.

**Culture of Sample**

Methods of the Japanese Society on Poultry Disease (1992) were used for the detection of SE in the organs, eggs, and feces of test samples.

**Detection of SE.** A 1-g sample of each organ was incubated in 10 mL of trypticase soy (TS) broth (E-MC13) at 37 C, and another 1-g sample was incubated in 10 mL of Hajna tetrathionate (HTT) broth medium (E-MH19) at 42 C for 24 h for the preculture of SE. The sample of eggshell was washed in 20 mL TS broth for 10 min, and then each wash broth was kept at 37 C for 24 h for the preculture. For preculture of sample egg yolks and whites, the same methods as described above were used. After part of the eggshell was removed, the egg white was removed aseptically using a sterilized pipette. The remaining egg yolk was also removed aseptically using a sterilized pipette. A 1-mL sample of egg yolk was incubated in TS broth medium, and another 1-mL sample was incubated in HTT broth. A 1-mL sample of egg white was incubated in TS broth, and another 1-mL sample was incubated in HTT broth.

For preculture of sample feces, the same methods described above were also used. A 1-g sample of feces was incubated in a TS broth, and another 1-g sample of feces was incubated in HTT broth.

A sample (0.1 mL) of each precultured broth media was plated on deoxycholate hydrogen sulfide lactose (DHL) agar (E-MA85) and was incubated at 37 C for 24 h.

**Counting of Enterobacteriaceae, Including SE, in Feces.** A 1-g sample of feces was suspended in 9 mL of sterilized physiological saline, and then the suspension was diluted from 10 to 10^7 times. A sample (0.1 mL) of each dilution was plated on DHL agar and was incubated at 37 C for 24 h. After incubation, Enterobacteriaceae, including SE colonies, were counted.

**Identification of SE on DHL Agar Medium.** The colonies on DHL agar were agglutinated with multivalent antiserum to Salmonella O-type (no. 211231) and with Salmonella O9-type (no. 211293) antiserum on a glass plate to identify the SE from other bacteria. A positive reaction was identified as SE.

**Statistical Analysis**

A chi-square test was used for all of the parameters, and Yates’s correction was practiced whenever it was necessary. To analyze the difference in the number of intestinal bacteria, Student’s t-test was used (Ichihara, 1990).

**Experiment 1: Inhibitory Effect of PHGG against SE Infection in Pullets**

A total of 125 9-wk-old White Leghorn pullets (Commercial Babcock B-305) was used in the study. They were divided into five experimental groups: 1-A, the control group; 1-B, the SE-infected group; 1-C-1, the group infected with SE and supplemented with 0.025% PHGG; 1-C-2, the group infected with SE and supplemented with 0.05% PHGG; and 1-C-3, the group infected with SE and supplemented with 0.1% PHGG. The commercial grower feed and water for 4 to 10-wk-old chicks were available to these groups ad libitum during the test period. The feed was supplemented with 0.025% PHGG, 0.05% PHGG, or 0.1% PHGG to Groups 1-C-1, 1-C-2, or 1-C-3, respectively.

At 10 wk of age the young hens of Groups 1-B, 1-C-1, 1-C-2, and 1-C-3 were inoculated orally by catheter with 3.2 × 10^6 cfu of SE per pullet. The bacterial strain of SE used was S. enteritidis IFO3313, which was donated by the IFO and was prepared to 3.2 × 10^6 cfu/mL and cultured overnight in TS broth. This strain was sent to the Institute for Fermentation from Statens Bakteriologiska Lab., Stockholm, Sweden, in 1954.

Feces were collected from each bird in its cage, in each experimental group, every day after having been infected by SE. Five pullets of each group were dissected on the day of SE infection. Birds were also dissected on the 3rd, 7th, 14th, and 21st d after SE infection. The liver, spleen, heart, ovaries, duodenum, small intestine, cecum, and large intestine were collected from these pullets to detect and identify SE.

Before dissection, sera were collected from five young hens on the 3rd, 7th, 14th, and 21st d after SE infection, and the agglutination titer against SE was determined using the microtiter method (Migita et al., 1995). The collected sera were diluted, and determination was made from samples diluted more than twice. An O-type antigen for determination of agglutination titer was prepared from S. enteritidis IFO3313 with the method reported by Nakamura et al. (1991).

**Experiment 2: Effect of PHGG on Intestinal Microflora in Pullets**

A total of 45 pullets, of the same species and age as in Experiment 1, were used in Experiment 2 to evaluate
intestinal microflora. The birds were divided into three experimental groups: 2-A, the control group; 2-B, the SE-infected group; and 2-C, the group infected with SE and supplemented with 0.025% PHGG. The feeding regimen provided by Zen-Noh Institute of Animal Health and the phage type was IV type.

Feces and eggs were collected every day after SE infection. A hen from each group was dissected on the day of SE infection. Two hens from each group on 3, 10, and 14 d after SE infection and four hens from each group 21 d after SE infection were also dissected. The liver, spleen, heart, ovary, ovarian follicle, duodenum, small intestine, cecum, and large intestine were collected from these hens to detect and identify SE.

### RESULTS

#### Experiment 1: Inhibitory Effect of PHGG against SE Infection in Pullets

The incidence of SE in the internal organs is shown in Table 2. There was no SE in any organs of any of the groups before infection or throughout the experiment for Group 1-A (control). *Salmonella enteritidis* was observed in many organs of all groups except Group 1-A 3 d after infection. Although SE was excreted gradually with the passage of time, it remained in a few organs of Groups 1-B (SE infected), 1-C-2 (0.05% PHGG), and 1-C-3 (0.1% PHGG) until 21 d after infection. In Group 1-C-1 (0.025% PHGG), SE disappeared entirely 14 d after infection.

The frequency of SE observed in organs during the entire test period was 26.7% in Group 1-B, 5.6% in Group 1-C-1, 14.4% in Group 1-C-2, and 17.8% in Group 1-C-3. It was significantly lower in Groups 1-C-1 (P < 0.01) and 1-C-2 (P < 0.05) and tended to decrease in Group 1-C-3 compared with Group 1-B.

### Experiment 2: Effect of Partially Hydrolyzed Guar Gum on Salmonella Infection

A total of 33 commercial ISA Brown laying hens, 72 wk of age, which were force-molted using the method reported by Otsuka (1987) at 69 wk of age, were used in this experiment. They were divided into three experimental groups: 1-A, the control group; 1-B, the SE-infected group; and 1-C, the group infected with SE and supplemented with 0.025% PHGG. A commercial feed for laying hens was supplemented to the feed. The laying hens of Groups 1-A (control) received 69 wk of age. For Group 1-B, 0.025% PHGG was supplemented to the feed. The laying hens of Groups 1-C-1, 1-C-2, and 1-C-3 were inoculated orally by the catheter with 3.4 × 10⁶ cfu of SE per hen at 73 wk of age. The bacterial strain of SE used was *S. enteritidis* ZK 2a, which was provided by Zen-Noh Institute of Animal Health and was prepared to 3.4 × 10⁶ cfu/mL with an overnight culture in TS broth. This bacterial strain was isolated from poultry, and the phage type was IV type.

Feces and eggs were collected every day after SE infection. A hen from each group was dissected on the day of SE infection. Two hens from each group on 3, 10, and 14 d after SE infection and four hens from each group 21 d after SE infection were also dissected. The liver, spleen, heart, ovary, ovarian follicle, duodenum, small intestine, cecum, and large intestine were collected from these hens to detect and identify SE.

#### Experiment 3: Inhibitory Effect of PHGG against SE Infection on Laying Hens

A total of 33 commercial ISA Brown laying hens, 72 wk of age, which were force-molted using the method reported by Otsuka (1987) at 69 wk of age, were used in this experiment. They were divided into three experimental groups: 3-A, the control group; 3-B, the SE-infected group; and 3-C, the group infected with SE and supplemented with 0.025% PHGG. A commercial feed for laying hens was supplemented to the feed. The laying hens of Groups 3-B and 3-C were inoculated orally by the catheter with 3.4 × 10⁶ cfu of SE per hen at 73 wk of age. The bacterial strain of SE used was *S. enteritidis* ZK 2a, which was provided by Zen-Noh Institute of Animal Health and was prepared to 3.4 × 10⁶ cfu/mL with an overnight culture in TS broth. This bacterial strain was isolated from poultry, and the phage type was IV type.

Feces and eggs were collected every day after SE infection. A hen from each group was dissected on the day of SE infection. Two hens from each group on 3, 10, and 14 d after SE infection and four hens from each group 21 d after SE infection were also dissected. The liver, spleen, heart, ovary, ovarian follicle, duodenum, small intestine, cecum, and large intestine were collected from these hens to detect and identify SE.

### Table 1. Culture media and methods used in Experiment 2

<table>
<thead>
<tr>
<th>Media</th>
<th>Main microorganisms enumerated</th>
<th>Dilution to be plated</th>
<th>Incubation time (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anaerobic bacteria¹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EG agar medium³</td>
<td>Predominant anaerobes</td>
<td>10⁶, 10⁷, 10⁸</td>
<td>3</td>
</tr>
<tr>
<td>BL agar medium</td>
<td>Predominant anaerobes</td>
<td>10⁶, 10⁷, 10⁸</td>
<td>3</td>
</tr>
<tr>
<td>BS agar medium</td>
<td><em>Bifidobacterium</em> spp.</td>
<td>10⁶, 10⁷, 10⁸</td>
<td>3</td>
</tr>
<tr>
<td>ES agar medium</td>
<td><em>Escherichia</em> spp.</td>
<td>10⁴, 10⁵, 10⁶</td>
<td>3</td>
</tr>
<tr>
<td>NBGT agar medium</td>
<td><em>Bacteroides</em> spp.</td>
<td>10¹, 10⁵, 10⁶</td>
<td>3</td>
</tr>
<tr>
<td>LBS agar medium</td>
<td><em>Lactobacillus</em> spp.</td>
<td>10¹, 10⁴, 10⁵</td>
<td>3</td>
</tr>
<tr>
<td>Aerobic bacteria</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DHL agar medium</td>
<td>Enterobacteriaceae</td>
<td>10¹, 10⁴, 10⁵</td>
<td>2</td>
</tr>
</tbody>
</table>

¹A sample (0.05 mL) of each dilution was plated on each medium in anaerobic globe box.

²Culture media were prepared and divided into petri dishes on a clean bench. Dishes were stored in an anaerobic chamber with mixed gas of 85% N₂, 10% CO₂, and 5% H₂ until plating. Plated bacteria were incubated in anaerobic chamber filled with CO₂ gas. The existence of O₂ gas was monitored with steel wool soaked with acidified copper sulfate solution.

³EG = eggert gagnon; Eiken Chemical Co., Ltd., Bunkyo-ku, Tokyo 113-8408, Japan. BL = glucose-blood-liver (E-MG16; Eiken Chemical Co., Ltd.); BS = *Bifidobacterium* spp. selective; ES = *Escherichia* spp. selective; NBGT = neomycin-brilliant green-taurocholate-bold; LBS = *Lactobacillus* spp. selective (11323; Becton Dickinson Microbiology Systems, Cockeysville, MD 21030); DHL = deoxycholate hydrogensulfide lactose (E-MA85; Eiken Chem. Co., Ltd.).

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Sakura, Chiba 285-0043, Japan.
The number and incidence of SE in the feces of pullets during the test period are shown in Figure 1. No SE from the feces was counted by direct culture on DHL agar medium, nor was SE observed in feces after preculture of TS or HTT broth for Group 1-A any test period. In all groups, except for Group 1-A, which was incubated by direct culture on DHL agar medium, SE was counted 10^3 to 10^6 cfu/g from the 1 to 3 d after infection. The SE was not counted before 4 d after infection for Groups 1-C-1 and 1-C-2 but was counted until 6 d after infection in Group 1-C-3. In Group 1-B, SE was counted until 7 d after infection (Figure 1a).

The incidence of SE in feces by preculture of either TS or HTT broth medium decreased rapidly and was not evident before 7 d after SE infection of Group 1-C-1. However, the incidence decreased gradually in Groups 1-B, 1-C-2, and 1-C-3 and was not detected before 13 d after SE infection in Group 1-C-3. The total incidence in feces during the whole period was 24.4% in Group 1-C-1, 28.8% in Group 1-C-2, and 35.6% in Group 1-C-3, whereas SE incidence was 60.0% in Group 1-B (Figure 1b).

The change of agglutinating O-type antibody titer to SE in serum is shown in Figure 2. The agglutinating antibody titer was significantly less in Groups 1-C-1, 1-C-2, and 1-C-3 than in Group 1-B.

**Experiment 2: Effect of PHGG on Intestinal Microflora in Pullets**

The change in bacterial counts of *Bifidobacterium* spp., *Lactobacillus* spp., *Eubacterium* spp., Bacteroidaceae, and Enterobacteriaceae and the total bacteria in the cecum of pullets is shown in Figure 3. Although there was no significant difference in the total bacterial population among the three groups (Figure 3f), the population of *Bifidobacterium* spp. (Figure 3a) and *Lactobacillus* spp. (Figure 3b) in Group 2-C (PHGG) was significantly greater than that in Groups 2-A (control) and 2-B (SE-infected) \((P < 0.05)\). There were no changes in Bacteroidaceae or *Eubacterium* spp. in the three groups (Figure 3c,d). However, the population of Enterobacteriaceae in Group 2-C was less than that in Group 2-B \((P < 0.05)\) on Day 14 (Figure 3e). Because SE is an Enterobacteriaceae, the increase in Enterobacteriaceae may indicate the increase of SE, and the decrease of Enterobacteriaceae may indicate the decrease of SE.

**Experiment 3: Inhibitory Effect of PHGG against SE Infection in Laying Hens**

The results of SE detection in various organs are shown in Table 3. There was no SE in any of the organs of any of the groups before SE infection or throughout the study in Group 3-A (Control). The SE was excreted gradually over time in Groups 3-B (SE infected) and 3-C (PHGG). *Salmonella enteritidis* was detected in many organs of Group 3-B, on Day 10 in all organs, on Day 14 in four organs, and on Day 21 in all organs. However, in Group 3-C, SE was detected only in a few organs on Day 10 in three organs, on Day 14 in three organs, and on Day 21 in four organs.

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**TABLE 2. Effect of administration of partially hydrolyzed guar gum (PHGG) to pullets on incidence of *Salmonella enteritidis* (SE) in various organs used in Experiment 1**

<table>
<thead>
<tr>
<th>Groups and organs(^1)</th>
<th>After infection(^2) (d)</th>
<th>SE observed in organs (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Group 1-A (control)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Internal organs</td>
<td>0/25(^1)</td>
<td>0/25</td>
</tr>
<tr>
<td>Intestine</td>
<td>0/20</td>
<td>0/20</td>
</tr>
<tr>
<td>Bird</td>
<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
<td>Group 1-B (SE infected)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Internal organs</td>
<td>0/25</td>
<td>1/25</td>
</tr>
<tr>
<td>Intestine</td>
<td>0/20</td>
<td>13/20</td>
</tr>
<tr>
<td>Bird</td>
<td>0/5</td>
<td>4/5</td>
</tr>
<tr>
<td>Group 1-C-1 (0.025% PHGG)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Internal organs</td>
<td>0/25</td>
<td>0/25</td>
</tr>
<tr>
<td>Intestine</td>
<td>0/20</td>
<td>4/20</td>
</tr>
<tr>
<td>Bird</td>
<td>0/5</td>
<td>2/5</td>
</tr>
<tr>
<td>Group 1-C-2 (0.05% PHGG)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Internal organs</td>
<td>0/25</td>
<td>1/25</td>
</tr>
<tr>
<td>Intestine</td>
<td>0/20</td>
<td>8/20</td>
</tr>
<tr>
<td>Bird</td>
<td>0/5</td>
<td>3/5</td>
</tr>
<tr>
<td>Group 1-C-3 (0.1% PHGG)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Internal organs</td>
<td>0/25</td>
<td>3/25</td>
</tr>
<tr>
<td>Intestine</td>
<td>0/20</td>
<td>8/20</td>
</tr>
<tr>
<td>Bird</td>
<td>0/5</td>
<td>3/5</td>
</tr>
</tbody>
</table>

\(^1\)Internal organs were liver, bile, spleen, heart, and ovary. Intestines were duodenum, small intestine, large intestine, and cecum.

\(^2\)The SE-positive samples/total samples observed.

**Significant \((P < 0.05)\) and \((P < 0.01)\) compared with Group 1-B.
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FIGURE 1. Number and incidence of SE in feces of pullets in Experiment 1. (a) Fecal suspension was incubated on deoxycholate hydrogen sulfide lactose (DHL) agar. Group 1-A (○; control), Group 1-B (●; SE infected), Group 1-C-1 (□; 0.025% partially hydrolyzed guar gum (PHGG)), Group 1-C-2 (▲; 0.05% PHGG), Group 1-C-3 (△; 0.1% PHGG). (b) Fecal suspension was precultured and incubated on DHL agar. Group 1-A (striped bar; control), Group 1-B (●; SE infected), Group 1-C-1 (□; 0.025% PHGG), Group 1-C-2 (checked bar; 0.05% PHGG), Group 1-C-3 (dotted bar; 0.1% PHGG). *,** Significant \( (P \leq 0.05 \text{ and } 0.01, \text{ respectively}) \) compared with Group 1-B.

The frequency of observing SE in organs during the entire test periods was 63.3% in Group 3-B and 16.7% in Group 3-C, which was statistically significant \( (P \leq 0.01) \).

The incidence of SE on the eggshell and in the egg white and egg yolk are shown in Table 4. On the eggshells, SE was not detected in Group 3-A during any of the test periods, whereas it was detected in Group 3-B from the 2 to 11 d and sporadically after 12 d of SE infection. Although SE was detected from 2 to 11 d, it was not detected before 12 d after SE infection in Group 3-C. The incidence of SE in all samples during the entire test was 12.5% in Group 3-C and 34.5% in Group 3-B, which was statistically significant \( (P \leq 0.01) \).

No SE was detected in the egg white or yolk in Groups 3-A or 3-C. In the egg white, SE was only detected 20 d after SE infection; in the egg yolk of Group 3-B, SE was detected on Days 7, 8, 16, and 20.

The SE in feces was not detected in either medium, which was incubated by direct cultivation on DHL agar and was precultured in HTT or TS broth medium in Group 3-A during the entire test period (Figure 4). The SE in Group 3-B was detected throughout most of the test period on the DHL agar medium by direct culture (Figure 4a). In Group 3-C, however, SE was detected from 2 to 11 d after SE infection. The SE was not detected in Group 3-C before 13 d in the HTT or TS broth medium by preculture (Figure 4b).

DISCUSSION

Feed supplemented with D-mannose has been reported to remarkably suppress the colonization of \( S. \) typhimurium in vitro (McHan et al., 1989; Oyofo et al., 1989c) and in vivo (DeLoach, 1989; Oyofo et al., 1989a). However, D-mannose is too expensive to be used in feed applications. Allen et al. (1997) have reported that palm kernel meal, which contains considerable amounts of structural carbohydrates such as arabinans, galactans, glucans, mannans, and pectins, is effective in preventing \( Salmonella \) spp. infection. However, as preparation on an industrial scale has not been established, it may be difficult to put this into practical use. Lactose has been reported to be effective in the prevention of \( S. \) typhimurium infection (Corrier et al., 1990; Ziprin et al., 1991; Tellez et al., 1993). However, it has also been suggested that the dosage of lactose used in this report causes diarrhea and decreases the daily gain.

FIGURE 2. Effect of feeding partially hydrolyzed guar gum (PHGG) on serum titer against \( Salmonella \) enteritidis (SE) in serum in Experiment 1. Group 1-A (○; control), Group 1-B (●; SE infected), Group 1-C-1 (□; 0.025% PHGG), Group 1-C-2 (▲; 0.05% PHGG), Group 1-C-3 (△; 0.1% PHGG). *,** Significant \( (P \leq 0.05 \text{ and } 0.01, \text{ respectively}) \) compared with Group 1-B.
In this study, we investigated the prevention of SE infection by the use of the carbohydrate, PHGG.

In Experiment 1, different dosages of PHGG (0.025, 0.05, and 0.1% in feed) were administrated to pullets to evaluate their preventive effect on the colonization of SE. Though SE was excreted gradually over time, PHGG apparently promoted a degree of SE excretion from organs (Table 2). The administration of PHGG resulted in decreases in the number and incidence of SE in feces (Figure 1). The agglutinating antibody titer to SE in serum also decreased (Figure 2). As a result, it is suggested that administration of PHGG promotes the excretion of SE from young birds, and the rate of transfer of SE to blood decreases.

In Experiment 1, of the feed supplemented with 0.025, 0.05, or 0.1% PHGG, that with 0.025% PHGG (Group 1-C-1) was the most effective in each index. This result suggests that for the prevention of SE infection, PHGG may not have a simple dose dependency, but an effective optimum dosage may exist.

Fukata et al. (1995) investigated the effects of inulo-, manno-, and fructooligosaccharide on resistance to *S. typhimurium* colonization in chicks. They reported that administration of 0.5 or 1.0% manno-oligosaccharide, which is the same type of PHGG, was weakly effective on resistance to *S. typhimurium* colonization. In our study, however, we found that administration of PHGG ranging from 0.025 to 0.1% was apparently effective in preventing SE infection. From this result, we expect that, because our dosage of PHGG in this study was much lower than that of manno-oligosaccharide in the study by Fukata et al., a dose lower than that used in their study would be effective. Thus, an effective optimum dosage might exist.

Imbalances in intestinal microflora sometimes induce physiological damage or different kinds of pathogenic diseases. These pathogenic diseases and physiological damage result in great losses from the livestock industry. In particular, in poultry the infection of SE is a serious

(Kogut et al., 1994). For these reasons, the development of a new, low-cost method for the prevention of SE infection may be important for maintaining public health.

**FIGURE 3.** Effect of feeding partially hydrolyzed guar gum (PHGG) on intestinal microflora in Experiment 2. Group 2-A (hatched bar; control), Group 2-B (SE infected), Group 2-C (partially hydrolyzed guar gum). *,**Significant \( (P \leq 0.05 \text{ and } 0.01, \text{ respectively}) \) compared with Group 2-A and 2-B. *a) Panel e. No significant difference was found with Group 2-A.

**TABLE 3.** Effect of administration of partially hydrolyzed guar gum (PHGG) to laying hens on incidence of *Salmonella enteritidis* (SE) in various organs used in Experiment 3

<table>
<thead>
<tr>
<th>Groups and organs(^1)</th>
<th>After infection(^2) (d)</th>
<th>SE observed in organs (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Group 3-A (control)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Internal organs</td>
<td>0/5</td>
<td>0/10</td>
</tr>
<tr>
<td>Intestine</td>
<td>0/4</td>
<td>0/8</td>
</tr>
<tr>
<td>Bird</td>
<td>0/1</td>
<td>0/2</td>
</tr>
<tr>
<td>Group 3-B (SE infected)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Internal organs</td>
<td>0/5</td>
<td>3/10</td>
</tr>
<tr>
<td>Intestine</td>
<td>0/4</td>
<td>8/8</td>
</tr>
<tr>
<td>Bird</td>
<td>0/1</td>
<td>2/2</td>
</tr>
<tr>
<td>Group 3-C (PHGG)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Internal organs</td>
<td>0/5</td>
<td>1/10</td>
</tr>
<tr>
<td>Intestine</td>
<td>0/4</td>
<td>3/8</td>
</tr>
<tr>
<td>Bird</td>
<td>0/1</td>
<td>2/2</td>
</tr>
</tbody>
</table>

\(^1\)Internal organs were liver, spleen, heart, ovary, and ovarian follicle. Intestines were duodenum, small intestine, large intestine, and cecum.

\(^2\)The SE-positive samples/total samples observed.

**Significant \( (P < 0.01) \) compared with Group 3-B.
TABLE 4. Effect of administration of partially hydrolyzed guar gum (PHGG) to laying hens on incidence of *Salmonella enteritidis* (SE) from egg used in Experiment 3

| Egg and groups | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 |
| After infection\(^1\) (d) Total incidence (%) | | | | | | | | | | | | | | | | | | | | | |
| Surface of egg-shell | | | | | | | | | | | | | | | | | | | | | |
| Group 3-A (control) | 0/3 | 0/4 | 0/3 | 0/2 | 0/1 | 0/2 | 0/1 | 0/2 | 0/1 | 0/2 | 0/1 | 0/2 | 0/1 | 0/2 | 0/1 | 0/2 | 0/1 | 0/2 | 0/1 | 0/2 | 0/3 | 0/1 |
| Group 3-B (SE infected) | 0/4 | 1/5 | 1/3 | 1/2 | 2/3 | 2/3 | 2/3 | 3/3 | 3/3 | 1/2 | 1/3 | 0/3 | 0/4 | 0/2 | 1/4 | 0/2 | 1/2 | 0/1 | 1/3 | 0/2 | 0/1 |
| Group 3-C (PHGG) | 0/3 | 1/4 | 1/2 | 1/2 | 0/2 | 0/2 | 1/3 | 0/4 | 2/4 | 0/2 | 1/4 | 0/4 | 0/4 | 0/2 | 0/3 | 0/2 | 0/1 | 0/3 | 0/2 | 0/2 | 0/1 |
| Egg white | | | | | | | | | | | | | | | | | | | | | |
| Group 3-A (control) | 0/3 | 0/4 | 0/3 | 0/2 | 0/1 | 0/2 | 0/1 | 0/2 | 0/1 | 0/2 | 0/4 | 0/3 | 0/4 | 0/2 | 0/3 | 0/1 | 0/3 | 0/2 | 0/2 | 0/3 | 0/1 |
| Group 3-B (SE infected) | 0/4 | 0/5 | 0/3 | 0/2 | 0/3 | 0/3 | 0/3 | 0/3 | 0/3 | 0/2 | 0/3 | 0/3 | 0/4 | 0/2 | 0/4 | 0/2 | 0/4 | 0/2 | 0/1 | 0/3 | 1/2 |
| Group 3-C (PHGG) | 0/3 | 0/4 | 0/2 | 0/2 | 0/2 | 0/2 | 0/3 | 0/4 | 0/4 | 0/2 | 0/4 | 0/4 | 0/2 | 0/3 | 0/2 | 0/1 | 0/3 | 0/2 | 0/2 | 0/1 |
| Egg yolk | | | | | | | | | | | | | | | | | | | | | |
| Group 3-A (control) | 0/3 | 0/4 | 0/3 | 0/2 | 0/1 | 0/2 | 0/1 | 0/2 | 0/1 | 0/2 | 0/4 | 0/3 | 0/4 | 0/3 | 0/2 | 0/3 | 0/1 | 0/3 | 0/2 | 0/3 | 0/1 |
| Group 3-B (SE infected) | 0/4 | 0/5 | 0/3 | 0/2 | 0/3 | 0/3 | 1/3 | 1/3 | 0/3 | 0/2 | 0/3 | 0/3 | 0/4 | 0/2 | 0/4 | 1/2 | 0/2 | 0/1 | 0/3 | 1/2 |
| Group 3-C (PHGG) | 0/3 | 0/4 | 0/2 | 0/2 | 0/2 | 0/2 | 0/3 | 0/4 | 0/4 | 0/2 | 0/4 | 0/4 | 0/2 | 0/3 | 0/2 | 0/1 | 0/3 | 0/2 | 0/2 | 0/1 |

\(^1\)The SE positive samples/total samples observed.

**Significant (P ≤ 0.05 and 0.01, respectively) compared with Group 3-B.

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**FIGURE 4.** Number and incidence of *Salmonella enteritidis* (SE) in feces of laying hens in Experiment 3. (a) Fecal suspension was incubated on deoxycholate hydrogen sulfoxide lactose (DHL) agar medium. Group 3-A (control), Group 3-B (SE infected), Group 3-C (PHGG). *,**Significant (P ≤ 0.05 and 0.01, respectively) compared with Group 3-B.
The effect of the administration of PHGG on laying hens in the prevention of contamination by SE on and in eggs was investigated in Experiment 3. The incidence of SE, on the eggshell and in the egg white and yolk suggests that administration of PHGG is effective in preventing SE infection (Table 4). Although the number of laying hens used in this experiment was small compared with that in Experiment 1, SE incidence in the organs of laying hens (Experiment 3) showed a similarity to that in pullets (Experiment 1) (Table 3).

The results in Figure 4 indicate that the number and incidence of SE in feces in Group 3-B were high during the entire test period. However, SE was not detected before Day 11 after infection when PHGG was administered. These results indicate that PHGG also has a preventive effect on SE infection in laying hens.

These results also indicate that administration of PHGG may have prevented contamination by SE in and on eggs by preventing SE infection in organs and the excretion of SE via the feces. Therefore, administration of PHGG to laying hens could control pollution in egg production.

It has been reported that administering PHGG to humans (Okubo et al., 1994) and rats (Takahashi et al., 1995) could increase the dominance of Bifidobacterium spp. and Lactobacillus spp., respectively, improve intestinal microflora balance and to stabilize the intestinal environment at a low pH. It was also found that the number of Bifidobacterium spp. and Lactobacillus spp. were increased by administering PHGG.

There are several studies in which D-mannose (Oyoo et al., 1980c; Droleskey et al., 1994) and mannose residue of oligosaccharide in lecin (Mirelman et al., 1989; Ofek and Beachey, 1980) were bound to the terminal of the sugar protein existing on the cell surface of pathogenic bacteria such as Salmonella spp., so that infected bacteria might be excreted with these carbohydrates from host animals (Ofek et al., 1977).

The PHGG is mainly composed of galactomannan, whose average molecular mass is about 20,000 Da, and the ratio of mannose and galactose is approximately 2:1 (Robinson et al., 1982; Englyst and Cummings, 1988). Our results suggest that PHGG has a preventive effect on SE infection by improving intestinal microflora balance and by an excreting effect on SE by binding with SE. In particular, it is suggested that the excreting effect on SE by binding with SE might contribute to low antibody titer to SE in serum.

It has been reported that the administration of fructooligosaccharide in combination with the competitive exclusion treatment is more effective in Salmonella infection (Bailey et al., 1991; Oyarzabal and Conner, 1996). It is suggested from their studies that administering PHGG with competitive exclusion may have a much greater effect.

Because the viscosity of PHGG, which is less than 13 mPa.s at 5°C in a 5% aqueous solution (Yamamoto et al., 1990) is low, it can also be used in drinking water.

The PHGG has been already used widely as a safe food ingredient (Takahashi et al., 1994b) (Sunfiber®, Taiyo Kagaku Co., Ltd., Japan). Therefore, because of its low cost, PHGG is an effective and useful material for large-scale applications, compared with other mannose derivatives, in the prevention of Salmonella infection in the poultry industry, especially in egg production.

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REFERENCES


EFFECT OF PARTIALLY HYDROLYZED GUAR GUM ON SALMONELLA INFECTION


