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## Evaluation of a Host-Specific Lactobacillus Probiotic in Neonatal Foals

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### ABSTRACT

A randomized, placebo-controlled, double-blind clinical trial was conducted on 54 neonatal foals to examine the effect and safety of a host-specific probiotic preparation. The probiotic contained a mixture of five strains of lactobacilli isolated from healthy horses (*Lactobacillus salivarius* YIT 0479, *L. reuteri* YIT 0480, *L. crispatus* YIT 0481, *L. johnsonii* YIT 0482 and *L. equi* YIT 0483), and was administered daily to 27 of the foals (1 to 7 days of age). A control group of 27 foals was given a placebo. Comparisons between the two groups were made for increase in body weight, the frequency of diarrhea, the composition of intestinal microflora, and the levels of short-chain fatty acids in the feces. Probiotic treatment caused no clinical side effects. The probiotic led to a significant increase ( $P < 0.01$ ) in body weight in 1-month-old foals and a significantly lower incidence ( $P < 0.05$ ) of diarrhea at 3 weeks of age. No significant differences were found between the fecal bacterial populations in the two groups, although a trend toward earlier colonization of *Lactobacillus* in the treated foals was seen. Our findings suggest that the administration of an equine-specific *Lactobacillus* probiotic to neonatal foals enhances growth and decreases the incidence of diarrhea.

### INTRODUCTION

The role of intestinal microflora in animal health and disease is of particular interest. Some bacterial species are clinically beneficial and when added to the diet as a food supplement are known to act as probiotics.<sup>1</sup> The live microorganisms beneficially affect the host by

improving its intestinal balance.<sup>2</sup> At present, the probiotics available for use in animals include those derived from the *Lactobacillus*, *Streptococcus*, *Enterococcus*, *Bacillus*, *Clostridium* and *Bifidobacterium* species.<sup>3</sup> In clinical trials, probiotics have been reported to enhance the growth of many domestic animals (including cows,<sup>4</sup> neonatal calves and piglets,<sup>5</sup> and broilers<sup>6</sup>) by improving the efficacy of forage digestion and by preventing and treating diarrhea.<sup>4,5</sup> Previous studies in horses have not shown any clinically important effects.<sup>7,8</sup> However, the bacterial strains used in the commercially available probiotic have not been disclosed.

In a study of the intestinal microflora of normal horses, we showed that the genus *Lactobacillus* is a predominant indigenous bacterium and that it produces lactic acid.<sup>9</sup> Furthermore, the stratified squamous epithelium of the nonsecreting area of the equine stomach was colonized by *Lactobacillus* species (*L. salivarius*, *L. crispatus*, *L. reuteri*, and *L. agilis*).<sup>10</sup> In vitro observations showed that these indigenous lactobacilli could attach host-specifically to the keratinized epithelial cells of the equine stomach.<sup>10</sup> We suggested that the indigenous lactobacilli were in a close symbiotic relationship with the host and contributed to the host's health. In another study, we isolated 178 strains of *Lactobacillus* species from the feces of yearlings and foals. They included *L. salivarius*, *L. johnsonii*, *L. crispatus*, *L. reuteri*, *L. plantarum*, *L. amylovorus*, *L. coryniformis*, and *Lactobacillus equi* species. Researchers proposed that this last strain be designated as a new species.<sup>11</sup>

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After the administration of an equine-specific probiotic to neonatal foals, the aim of the present study was to evaluate the effects of early colonization of Lactobacilli species in the intestine on body weight, fecal characteristics, and the occurrence of diarrhea.

## MATERIALS AND METHODS

### Preparation of Probiotic

The probiotic (designated as S5M) was produced by lyophilizing one strain of each of five species of Lactobacillus (*L. salivarius* YI 0479, *L. reuteri* YIT 0480, *L. crispatus* YIT 0481, *L. johnsonii* YIT 0482, and *L. equi* YIT 0483). Each strain had been isolated from either the equine feces or the gastric epithelium, and in previous *in vitro* studies was highly adherent to cells in the digestive tract. 10 Skim milk and trehalose were used as lyophilization protectors. After lyophilization, the number of viable bacteria of each strain was counted using the CFU-enumerating method. The number of each strain was adjusted to more than  $4 \times 10^8$  CFU/g using starch as a diluting agent, and a dose of 5 g of the mixed preparation containing  $1$  to  $4 \times 10^{10}$  viable bacteria was used. A placebo was prepared by substituting starch for the bacteria.

### Animals

The study was approved by the Animal Care and Use Committee of the Japan Bloodhorse Breeder's Association. A randomized, double-blind, placebo-controlled study was designed. Thirty-two thoroughbred foals, born between March and June 2002 in Hokkaido (5 breeding farms) and 22 thoroughbred foals born in Kagoshima prefecture (7 breeding farms) were studied. At each farm, equal numbers of foals were given the probiotic (treated group) and the placebo (control group). The animals were randomly assigned to each group, and the researchers were blinded to the assignments until the end of the study.

### Administration of Probiotic and Placebo

In the treated group, 5 g probiotic dissolved in 50 mL of 5% glucose solution was given orally via a syringe 20 hours after birth, and then daily until 7 days of age. This time period was chosen because it corresponds to the moment of highest risk of gastrointestinal diseases in foals.<sup>12</sup> The placebo was administered in an identical manner to the treated group of foals.

### Clinical Observations

**Body weight:** Body weight (in kilograms) was measured on five occasions (at birth and at 7, 14, 21, and 30 days of age) using a digital walk-on scale.

**Fecal characteristics:** Up to 30 days of age, the consistency of the feces of each foal was assessed using the following scoring scale: (normal), 1 (soft), 2 (semi-solid nonformed), or 3 (watery diarrhea). Scores of 2 and 3 were indicative of diarrhea. In addition, a full clinical record was kept for each foal, including details of the treatment given for the diarrhea.

### Microflora Analysis

**Collection and transportation of fecal samples:** A fecal sample was collected from the rectums of 12 foals (1, 2, 3, 7, and 14 days of age); 5 foals were randomly selected from both the treated and control groups. Each sample was placed in a 50-mL plastic centrifuge tube, and the tubes were then collated in vinyl bags containing a deoxidizer (Anaeropack, Mitsubishi Gas Chemical, Tokyo, Japan). The vinyl bags were closed with clips, placed in an ice-box, and transported within two days to the laboratory. Our previous study confirmed no detectable changes in the composition of microflora and short chain fatty acids in the fecal samples during transportation.<sup>9</sup>

**Microflora analysis:** The number of viable bacteria in 1 g of feces was calculated using a previously described method.<sup>13-15</sup> A mortar and pestle were used to make a 10% solution of each fecal sample in an anaerobic diluent. Then, 200 mL of the solution was added to 1.8-mL anaerobic diluent under carbon dioxide, and tenfold dilutions were repeated until a 10<sup>7</sup>-fold dilution of the original solution was attained. The final solution was inoculated onto various culture media (Table 1) and the number of viable bacteria per gram of feces was calculated from the number of resultant colonies.

### Measurement of Short-Chain Fatty Acids

To examine the metabolic activity of microflora, part of each fecal sample was dissolved in the anaerobic diluent to provide a 10% fecal solution. In the study, 100 mL of a solution containing an equal amount of 100 mM crotonic acid and 10% perchloric acid was added to 400 mL of each fecal solution, and the mixture was stored at 4°C. Measurement of short-chain fatty acids (SCFAs) (acetic, propionic, butyric, valeric, isobutyric, isovaleric, lactic, and succinic acid) was performed using HPLC according to our previous method.<sup>13</sup>

#### Hematologic and Biochemical Studies

Blood samples were collected from each foal at 1, 7, and 14 days and 1, 2, and 3 months of age. Blood cell (erythrocyte, leucocyte, eosinophil, neutrophil, lymphocyte, monocyte, and platelet) counts were determined. Other parameters measured were hemoglobin and hematocrit, as well as serum levels of bilirubin, alkaline phosphatase, glutamic-oxaloacetic transaminase, glutamic-pyruvic transaminase, lactate dehydrogenase, creatine phosphokinase, total protein, albumin, a-, b-, and g-globulin, total cholesterol, high-density lipoprotein cholesterol, blood urea nitrogen, chloride, sodium, and potassium. The hematologic analyses were performed using an automated hematology analyzer (Sysmex SE-9000, Sysmex Corporation, Kobe, Japan) and the biochemical analyses using chemistry immunoanalyzers (Olympus AU5200 and AES630, Olympus Optical, Tokyo, Japan).

#### Statistical Analyses

Statistical analyses (SAS System Release 6.12, SAS Institute, Cary, NC) were performed to look for evidence of significant differences between the treated and control groups of foals. Body weight was analyzed using a repeated-measures analysis of variance (ANOVA), and pairwise comparisons were corrected with the Bonferroni correction for multiple testing. The incidence of diarrhea, medical treatment, and use of antibiotics were analyzed at each week of age using Fisher's exact probability test (2-tailed) with the Bonferroni correction for multiple testing. A value of  $P < 0.05$  was considered statistically significant.

### RESULTS

#### Clinical Observations

**Body weight:** The probiotic caused a significant overall increase ( $P = 0.008$ ) in the body weight of foals (Table 2). Initially (1 and 7 days of age), no significant differences were seen between the two groups. However, the body weight of the treated foals was significantly greater than that of the control animals at 14, 21, and 30 days of age ( $P < 0.05$ ).

**Fecal characteristics:** At 2 to 3 weeks of age, the incidence of diarrhea was significantly ( $P < 0.05$ ) lower (14.8%) in the treated foals than in the control animals (51.9%). None of the treated foals were given medical treatment or antibiotics at 3 to 4 weeks of age (Table 3).

#### Composition of Intestinal Microflora

At up to 2 weeks of age, no significant differences were found between the treated and control animals in terms of the number of individual bacterial species in the fecal samples (Table 4). We saw a tendency for earlier colonization of *Lactobacillus* in the intestinal tract in the treated foals. At 3 days of age, *Lactobacillus* was detected in 83.3% of the treated foals and in 60% of the control animals; the results at 5 days of age were 100% and 66.7%, respectively.

#### Short-Chain Fatty Acids

The concentration of total SCFAs and of individual SCFAs in the fecal samples from foals up to 14 days of age are given in Table 5. The SCFAs detected were mainly acetic acid, propionic acid, butyric acid, and lactic acid, each of which is produced by *Lactobacillus*. Only negligible amounts of isobutyric, valeric, isovaleric, and succinic acid were recorded. We saw no statistically significant differences between the two groups, apart from a significantly greater concentration ( $P < 0.05$ ) of total SCFAs at 7 days of age in the treated foals.

#### Hematologic and Biochemical Studies

In comparing the treated and control groups, the probiotic had no statistically significant effect on the hematologic and biochemical parameters.

### DISCUSSION

There is an increasing awareness of the importance of intestinal microflora in the health and disease of domestic animals, and strategies have been developed to promote health by manipulating the microflora. Antibiotics have long been used to promote the health and growth of animals. Concern is growing, however, that the continued use of antibiotics will result in the proliferation and spread of antibiotic-resistant bacteria. Researchers have also reported that some antibiotics induce severe colitis in ponies.<sup>16</sup> We believe, however, that it is appropriate to supplement the diet of neonatal foals with probiotics containing only normal equine microorganisms that will ultimately colonize the intestinal tract.

Of particular interest in this study was the increased body weight of the treated foals at 2 to 4 weeks of age. This beneficial effect of the probiotic was thought to result in part from improved intestinal function, because a reduced incidence of diarrhea was found in the treated group. Diarrhea in neonatal foals is of great clinical importance because of the associated mortality, the high costs of management and treatment, and the high incidence of other clinical complications. At 7 to 10 days of age, foals often develop diarrhea ("foal heat diarrhea") coincident with the mare's first estrous after foaling.<sup>17</sup> The etiology is still not fully understood. Researchers originally thought that changes in the composition of the mare's milk, occurring during the mare's estrus cycle, could have resulted in the foal's diarrhea. Such an association has been questioned after an investigation showed no correlation between the occurrence of diarrhea in foals and the ingredients of their mare's milk.<sup>18</sup> Furthermore, an apparently similar form of diarrhea also occurs in bottle-fed foals.

Masri et al.<sup>19</sup> have reported that the diarrhea could be caused by the hypersecretion of electrolytes from the mucosa of the small intestinal and may simply be related to a normal developmental change in the gastrointestinal tract. Researchers believe that diarrhea at 2 to 4 weeks of age may follow an instability in the intestinal microflora that continues from foal heat diarrhea. In this study, the probiotic led to an earlier recovery from foal heat diarrhea, perhaps by enhancing establishment of the normal intestinal microflora. This idea is supported by the fact that there was a tendency for earlier colonization of *Lactobacillus* in the treated foals than in the control group.<sup>20,21</sup>

The gastrointestinal tract is sterile at birth, but is colonized rapidly. In healthy neonatal foals, a well-defined sequence of colonization occurs. Facultative anaerobes appear first, followed by strict anaerobes (*Bacteroidaceae* species) and indigenous lactic acid bacteria (*Lactobacillus* species), which predominate by 2 weeks of age.<sup>9</sup> The metabolic consequences of this colonization result in the production of SCFAs in the colon. The total SCFAs in the feces at 7 days of age in the treated group was significantly higher than that in the control group. SCFAs are important as they are the preferred energy source of the colonic epithelium and stimulate sodium and water absorption from the colon.<sup>22</sup>

In conclusion, the administration of a probiotic to neonatal foals was not associated with any clinical side effects and promoted the animals' growth and intestinal health. Future studies could be aimed at investigating the efficacy of probiotics for the treatment of diarrhea in adult horses.

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#### REFERENCES

1. Lee Y-K, Nomoto K, Salminen S, et al: Handbook of probiotics. Lee Y-K, ed. New York: John Wiley & Sons, 1999.
2. Fuller E: Probiotics in man and animal. *J Appl Bact* 66:365–378, 1989.
3. McCullough ME: Do Probiotics have a role in our dairy ration? *Hoard's Dairyman* 133:25, 1988.
4. Doreau M, Jouany JP: Effect of a *Saccharomyces cerevisiae* culture on nutrient digestion in lactating dairy cows. *J Dairy Sci* 81:3214–3221, 1998.
5. Kyriakis SC, Tsiloyiannis VK, Vlemmas J, et al: The effect of probiotic LSP 122 on the control of post-weaning diarrhoea syndrome of piglets. *Res Vet Sci* 67:223–228, 1999.
6. Tellez G, Petrone VM, Escorcía M, et al: Evaluation of avian-specific probiotic and *Salmonella enteritidis*-, *Salmonella typhimurium*-, and *Salmonella heidelberg*-specific antibodies on cecal colonization and organ invasion of *Salmonella enteritidis* in broilers. *J Food Prot* 64:287–291, 2001.
7. Kim LM, Morley PS, Traub-Dargatz JL, et al: Factors associated with *Salmonella* shedding among equine colic patients at a veterinary teaching hospital. *J Am Vet Med Assoc* 218:740–748, 2001.
8. Parraga ME, Spier SJ, Thumond M, Hirsh D: A clinical trial of probiotic administration for prevention of salmonella shedding in the postoperative period in horses with colic. *J Vet Intern Med* 11:36–41, 1997.
9. Sakaitani Y, Yuki N, Nakajima F, et al: Colonization of intestinal microflora in newborn foals. *J Intest Microbiol* 13:9–14, 1999.