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# EFFECT OF TOYOCERIN POWDER® (BACILLUS TOYOI) ON THE INTESTINAL BACTERIAL FLORA OF RABBITS

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## INTRODUCTION

During the last decade a renewed interest has been shown on the role and effect of probiotics in animal production (Crawford, 1979; Savage, 1981; Miles, 1981; Tahir et al., 1983). The extensive research and field works conducted with TOYOCERIN POWDER® in several animal species (Kozasa, 1978) has shown Bacillus toyoi, the active principle, a remarkable agent in improving the performance of poultry, swine and ruminants. Enteritis is a major disease problem of rabbits (Grobner,1982). The etiology and pathogenesis of this enteritis complex syndrome are not clearly understood, although bacterial organisms have been incriminated as one of the main agents of this disease (Patton, et al., 1978). Since probiotics increase the number of beneficial organisms while decreasing the number of pathogenic organisms of the gastrointestinal tract, the purpose of this work was to study the effect of B. toyoi on the gastrointestinal tract microflora of the rabbit. Routine observations of growth performance, diarrhea and other clinical signs were also carried out.

## MATERIAL AND METHODS

A total of eighty-three Japanese White rabbits 33-35 days old (average weight, 722.0 ± 71.7 g) were used. After a three days acclimation period with TOYOCERIN-free feed upon arrival, 80 rabbits were randomly alloted to four groups of 20 rabbits each. The remaining animals were killed at the initiation of the trial and subjected to bacteriologic examination of gastrointestinal contents. Feed and water were available ad libitum throughout the study. Rabbits were housed in metal cages, 5 to each cage, with rice straw as litter. The straw bedding was replaced every third or fourth day. The diet fed was a commercial rabbit feed (CR-1, CLEA, Japan Inc.). The diet analysis data are shown in Table 1.
TOYOCERIN POWDER (Batch No ETBC-3002) was added to the feed at concentrations of $1x10^5$, $1x10^6$ and $5x10^6$ spores/g. The feed was mixed and pelleted. The same feed without TOYOCERIN POWDER was also pelleted and fed to control rabbits. The experimental groups were as followed:

Group 1 . . . . . . . . Control
Group 2 . . . . . . . . $1x10^5$ B.toyoi spores/g feed.

Group 3 . . . . . . . $1x10^6$ B.toyoi spores/g feed.
Group 4 . . . . . . . $5x10^6$ B.toyoi spores/g feed.

The feeding period was 21 days. Body weights of individual animals were recorded at the beginning of the acclimation period, and at 0, 1, 2 and 3 weeks after initiation of trial.Three rabbits from each group were killed at each period and were subjected to bacteriologic examination of intestinal contents. All rabbits were observed daily for clinical signs throughout the trial.

Table 1. Diet Analysis

| | |
|---|---|
| Crude Protein | 18.5 % |
| Crude Fat | 3.1 % |
| Crude Fiber | 11.8 % |
| Ash | 7.3 % |
| Nitrogen-free Extract | 51.3 % |
| Energy, Calculated | 307.1 Cal. |

Enterobacteriacea and B.toyoi count from stomach, duodenum, jejunum, ileum, cecum and rectum were performed. In addition, total bacteria and Lactobacillus, Bifidobacterium, Staphylococcus, Clostridium and Bacteroides count was also carried out. Isolation, identification and counting of bacteria were performed according to Mitsuoka (1980). The bacterial count was expressed as number-logarithm-of organisms per gram of alimentary content.

## RESULTS

Body weight and clinical signs. TOYOCERIN POWDER caused a consistent and steady increase in body weight. A summary of the growth data is shown on Table 2. From about one week after initiation of the trial, nearly 50% of rabbits in the Control Group showed evidence of severe watery diarrhea. Five rabbits in this group eventually died. Signs of diarrhea were seen in Groups 2 and 3, although the symptoms were generally milder in these animals. Three animals died in Group 2 and only a single rabbit fatality ocurred in Group 3.A few rabbits in Group 4 were found to have wet tails after one week on trial, but they showed later no aggravation of the symptoms and no mortality. Mortality rates are shown in Table 3.

Bacteriologic examination. a) Bacillus toyoi. B.toyoi was not present in any of the gastrointestinal regions examined in Group 1 (Control) throughout the trial. In Group 2 ($1x10^5$ spores/g), an almost constant number of organisms was detected in all regions at all periods - approximately $10^2$-$10^3$ cells/g in the stomach, duodenum, jejunum and ileum, and $10^4$-$10^5$ cells /g in the cecum and rectum. In Group 3 ($1x10^6$ spores/g), the organism was recovered from all specimens at all periods - $10^3$ -$10^4$ cells/g in stomach, duodenum, jejunum and ileum, and $10^5$-$10^6$ cells/g in the cecum and rectum. In Group 4 ($5x10^6$ spores/ g), B.toyoi was recovered from all specimens at all periods as in Group 3. It was interesting to observe that the cecum and rectum contained 10-100 fold as compared to the small intestine in all individuals.

Table 2. Mean Body Weight (g)

| Group | 0 Week | 1 Week | 2 Weeks | 3 Weeks |
|---|---|---|---|---|
| 1 | 773 ± 88 (20) | 981 ± 136 (20) | 1245 ± 188 (16) | 1350 ± 216 (9) |
| 2 | 771 ± 81 (20) | 983 ± 121 (20) | 1272 ± 158 (17) | 1458 ± 240 (11) |
| 3 | 773 ± 84 (20) | 1003 ± 121 (20) | 1312 ± 133 (17) | 1602 ± 174 (13) |
| 4 | 774 ± 82 (20) | 1022 ± 127 (20) | 1346 ± 136 (17) | 1649 ± 129 (14) |

( ): Number of Rabbits (Except killed and dead animals).

Table 3. Mortality rates

| Group | 0 ~ 1 Week | 1 ~ 2 Weeks | 2 ~ 3 Weeks | 0 ~ 3 Weeks |
|---|---|---|---|---|
| 1 | 0/20 | 1/17 | 4/13 | 5/20 |
| 2 | 0/20 | 0/17 | 3/14 | 3/20 |
| 3 | 0/20 | 0/17 | 1/14 | 1/20 |
| 4 | 0/20 | 0/17 | 0/14 | 0/20 |

b) Escherichia coli. Data on the E.coli count are shown in Table 4. At the beginning of the trial about $10^2$ organisms/g were only detected in some of the regions of the alimentary tract of one of the three rabbits examined. In Group 1, E.coli was demonstrated in nearly all specimens at all periods, the population tending to increase with descending site of the alimentary tract. Some animals showed at 3 weeks as many as $10^8$-$10^9$ bacteria/g in the cecum and rectum. In Group 2, some specimens revealed slightly smaller E.coli populations at 1-2 weeks as compared to Group 1. However, at 3 weeks $10^5$-$10^8$ cells/g were recovered from all regions as in Group 1. In Groups 3 and 4, E.coli was recovered at $10^3$-$10^4$ cells/g, while some rabbits showed populations of about $10^5$-$10^6$ cells/g in the lower alimentary tract. It was frequent to find no E.coli in all regions examined in these two Groups.
c) Other bacteria. Data on total bacteria count and other bacteria are shown in Table 5. Lactobacillus, Bifidobacterium and Clostridium were not isolated from any of the specimens examined. Streptococcus and Staphylococcus were only sporadically detected at about $10^2$-$10^4$ cells/g. Bacteroides was found to be the most abundant organism, greatly contributing to the intestinal microbiota; it was recovered in quantities of $10^4$-$10^8$ cells/g from the duodenal contents, and $10^8$-$10^9$ cells/g from the cecal contents.

## DISCUSSION

A high incidence of diarrhea during the first 2 weeks in Group 1 (Control) and Group 2 ($1 \times 10^5$ spores/g) was shown in this trial. Some of the rabbits in these groups eventually died of dehydration. A high incidence of E.coli was demonstrated in the alimentary tract of animals from Group 1 and 2, thus suggesting that E. coli might play an important role in the pathogenesis of diarrhea. There was a delay in the development of diarrhea, with much lower mortality, and notably lower E.coli count in intestines from Groups 2 and 3 receiving higher concentrations of B.toyoi ($1 \times 10^6$ - $5 \times 10^6$ spores/g). These findings suggest that B.toyoi exerted a suppressive effect directly or indirectly on E.coli proliferation in the rabbit alimentary tract. These data confirm previous results (Kozasa, 1978) that showed that B.toyoi, when administered orally, suppressed the growth of pathogenic bacteria, specially enterotoxigenic E.coli, in the intestinal tract of piglets and calves.
It was found in the cecum 10 to 100 fold B.toyoi increase over the upper alimentary tract. The cause underlying this phenomenon is yet to be elucidated.
An interesting finding was the absence of clostridial organisms in rabbits from all groups. Although it has been reported (Grobner, 1982) the isolation of Clostridium perfringens and several other clostridial organisms from healthy and enteritic rabbits, we were unable to isolate such organisms in this trial.
The growth response to increasing levels of B.toyoi in feed seems to reflect the inhibitory effect of this organism on diarrhea in rabbits.
The specimens were also examined for changes in intestinal populations of Lactobacillus and Bifidobacterium which are general

**Table 4. Escherichia coli count**

| | | | | | |
|---|---|---|---|---|---|
| 0 Week | Stomach | 0 ( 0 ) | | | |
| | Duodenum | 0 ( 0 ) | | | |
| | Jejunum | 2.6 ( 1 ) | | | |
| | Ileum | 2.5 ( 1 ) | | | |
| | Caecum | 2.7 ( 1 ) | | | |
| | Rectum | 0 ( 0 ) | | | |
| | Group | 1 | 2 | 3 | 4 |
| 1 Week | Stomach | 3.8 ( 1 ) | 3.1 ( 1 ) | 2.2 ( 1 ) | 2.2 ( 1 ) |
| | Duodenum | 3.4±0.7* ( 3 ) | 3.4 ( 1 ) | 3.1 ( 1 ) | 2.8 ( 1 ) |
| | Jejunum | 4.2±1.1 ( 3 ) | 2.6±0.8 ( 3 ) | 2.7±0.8 ( 2 ) | 3.1 ( 1 ) |
| | Ileum | 4.8±1.5 ( 3 ) | 4.1 ( 1 ) | 2.8±0.8 ( 2 ) | 3.0±0.3 ( 2 ) |
| | Caecum | 4.9±1.3 ( 3 ) | 3.6±1.4 ( 3 ) | 3.5±0.5 ( 2 ) | 3.6±1.0 ( 2 ) |
| | Rectum | 4.2±1.3 ( 3 ) | 3.4±1.4 ( 3 ) | 3.3±0.6 ( 2 ) | 4.0 ( 1 ) |
| 2 Weeks | Stomach | 3.5 ( 1 ) | 2.7 ( 1 ) | 3.1 ( 1 ) | 2.1 ( 1 ) |
| | Duodenum | 4.7±3.2 ( 3 ) | 4.1 ( 1 ) | 3.1±1.3 ( 2 ) | 0 ( 0 ) |
| | Jejunum | 5.0±3.2 ( 3 ) | 3.8±1.0 ( 3 ) | 2.9±0.8 ( 3 ) | 3.3 ( 1 ) |
| | Ileum | 6.0±1.6 ( 2 ) | 4.1±1.4 ( 2 ) | 3.5±0.6 ( 2 ) | 2.4±0.6 ( 3 ) |
| | Caecum | 6.2±2.4 ( 3 ) | 4.4±1.3 ( 3 ) | 3.5±1.4 ( 3 ) | 3.0±1.2 ( 3 ) |
| | Rectum | 6.2±2.8 ( 3 ) | 3.8±1.7 ( 3 ) | 2.9±1.1 ( 3 ) | 3.1±1.2 ( 3 ) |
| 3 Weeks | Stomach | 3.2 ( 1 ) | 3.1 ( 1 ) | 3.2 ( 1 ) | 3.4 ( 1 ) |
| | Duodenum | 5.6±2.2 ( 3 ) | 5.2±1.8 ( 3 ) | 2.8±1.0 ( 3 ) | 2.4 ( 1 ) |
| | Jejunum | 5.3±1.9 ( 3 ) | 5.3±1.2 ( 3 ) | 3.5±1.9 ( 2 ) | 3.2±1.4 ( 2 ) |
| | Ileum | 5.3±1.9 ( 3 ) | 6.0±1.9 ( 3 ) | 5.1 ( 1 ) | 3.9±0.8 ( 3 ) |
| | Caecum | 7.8±1.4 ( 3 ) | 7.9±1.6 ( 3 ) | 3.8±1.9 ( 3 ) | 3.9±2.0 ( 3 |
| | Rectum | 8.2±0.8 ( 3 ) | 7.6±1.2 ( 3 ) | 3.6±1.5 ( 3 ) | 3.6±1.5 |

* : Average bacterial count ±SD

( ) : Number of detectable animal

0 : <Assay limit

Table 5. Total bacterial and other bacteria count

| Group | Week | | Total bacterial count | Lactobacillus | Bifidobacterium | Streptococcus | Staphylococcus | Clostridium | Bacteroides |
|---|---|---|---|---|---|---|---|---|---|
| | Initial | Duodenum | 6.8 ± 2.1 | 0 (0) | 0 (0) | 0 (0) | 2.1 (1) | 0 (0) | 6.6 ± 2.1 (3) |
| | | Caecum | 9.2 ± 0.2 | 0 (0) | 0 (0) | 3.4 (1) | 0 (0) | 0 (0) | 9.1 ± 0.3 (3) |
| 1 | 1 | Duodenum | 8.5 ± 0.9 | 0 (0) | 0 (0) | 2.8 (1) | 0 (0) | 0 (0) | 8.4 ± 0.9 (3) |
| | | Caecum | 9.2 ± 0.3 | 0 (0) | 0 (0) | 3.2 ± 1.3 (2) | 0 (0) | 0 (0) | 9.1 ± 0.3 (3) |
| | 2 | Duodenum | 7.1 ± 0.6 | 0 (0) | 0 (0) | 0 (0) | 3.4 (1) | 0 (0) | 6.9 ± 1.4 (3) |
| | | Caecum | 9.2 ± 0.4 | 0 (0) | 0 (0) | 3.3 ± 0.1 (2) | 0 (0) | 0 (0) | 9.1 ± 0.4 (3) |
| | 3 | Duodenum | 6.7 ± 1.4 | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 6.7 ± 1.4 (3) |
| | | Caecum | 9.3 ± 0.5 | 0 (0) | 0 (0) | 4.5 ± 1.7 (3) | 0 (0) | 0 (0) | 9.1 ± 0.2 (3) |
| 2 | 1 | Duodenum | 5.7 ± 1.3 | 0 (0) | 0 (0) | 0 (0) | 3.2 (1) | 0 (0) | 5.6 ± 1.4 (3) |
| | | Caecum | 8.8 ± 0.7 | 0 (0) | 0 (0) | 3.5 ± 1.0 (2) | 0 (0) | 0 (0) | 8.7 ± 0.8 (3) |
| | 2 | Duodenum | 8.2 ± 0.6 | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 8.0 ± 0.6 (3) |
| | | Caecum | 8.6 ± 0.6 | 0 (0) | 0 (0) | 5.2 (1) | 2.5 (1) | 0 (0) | 8.6 ± 0.7 (3) |
| | 3 | Duodenum | 5.9 ± 1.6 | 0 (0) | 0 (0) | 3.0 (1) | 0 (0) | 0 (0) | 5.1 ± 0.9 (3) |
| | | Caecum | 9.1 ± 0.4 | 0 (0) | 0 (0) | 3.6 ± 0.8 (2) | 0 (0) | 0 (0) | 8.9 ± 0.3 (3) |
| 3 | 1 | Duodenum | 6.1 ± 1.2 | 0 (0) | 0 (0) | 0 (0) | 2.6 ± 0.2 (2) | 0 (0) | 6.0 ± 1.2 (3) |
| | | Caecum | 9.3 ± 0.3 | 0 (0) | 0 (0) | 3.0 (1) | 2.5 (1) | 0 (0) | 9.3 ± 0.4 (3) |
| | 2 | Duodenum | 6.2 ± 1.0 | 0 (0) | 0 (0) | 2.0 (1) | 0 (0) | 0 (0) | 6.2 ± 1.0 (3) |
| | | Caecum | 8.5 ± 0.6 | 0 (0) | 0 (0) | 3.4 (1) | 3.1 (1) | 0 (0) | 8.5 ± 0.6 (3) |
| | 3 | Duodenum | 6.2 ± 1.7 | 0 (0) | 0 (0) | 0 (0) | 2.8 (1) | 0 (0) | 6.2 ± 1.6 (3) |
| | | Caecum | 9.0 ± 0.4 | 0 (0) | 0 (0) | 3.2 ± 0.5 (2) | 0 (0) | 0 (0) | 9.0 ± 0.4 (3) |
| 4 | 1 | Duodenum | 5.5 ± 1.5 | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 5.5 ± 1.5 (3) |
| | | Caecum | 9.1 ± 0.2 | 0 (0) | 0 (0) | 3.1 (1) | 0 (0) | 0 (0) | 9.1 ± 0.2 (3) |
| | 2 | Duodenum | 7.4 ± 1.1 | 0 (0) | 0 (0) | 0 (0) | 3.1 (1) | 0 (0) | 7.3 ± 1.1 (3) |
| | | Caecum | 9.3 ± 0.6 | 0 (0) | 0 (0) | 0 (0) | 2.4 ± 0.1 (2) | 0 (0) | 9.3 ± 0.6 (3) |
| | 3 | Duodenum | 5.6 ± 1.4 | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 5.5 ± 1.4 (3) |
| | | Caecum | 9.0 ± 0.4 | 0 (0) | 0 (0) | 3.4 (1) | 0 (0) | 0 (0) | 9.0 ± 0.4 (3) |

( ) : Number of detectable animal

ly regarded as favorable organisms in the normal intestinal microflora on other animal species. But, it was another interesting finding, neither organism was isolated from the alimentary tract in this study. These data do not confirm the findings of Smith and Crabb (1961).

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## SUMMARY


TOYOCERIN POWDER®, a viable bacterial preparation containing as the active principle spores of Bacillus toyoi, was administered in feed to study its effect on the gastrointestinal tract microflora of the rabbit. Increasing amounts of B.toyoi ($1x10^5$, $1x10^6$ and $5x10^6$ spores/g feed) caused a significant reduction and complete prevention of diarrhea in rabbits, and a remarkable favorable effect on body weight. A significant decrease in Escherichia coli count was found in animals consuming $1x10^6$ and $5x10^6$ spores/g feed, confirming the inhibitory effect of B.toyoi on enterotoxigenic E.coli in piglets and calves. These results show that the feeding of viable bacterial products can be an effective way of preventing rabbit dysentery.


## RESUMEN


TOYOCERIN POWDER®, un producto a base de bacterias viables que contiene como principio activo esporas de Bacillus toyoi, fué administrado en el pienso para estudiar su efecto sobre la microflora del tracto gastrointestinal del conejo. Niveles crecientes de B.toyoi ($1x10^5$, $1x10^6$ y $5x10^6$ esporas/g de pienso) produjeron una reducción significativa y la prevención comple-

ta de diarrea en los conejos, y un marcado efecto favorable sobre el peso corporal. Se observó un descenso significativo en el contaje de *Escherichia coli* en animales que consumieron $1x10^6$ y $5x10^6$ esporas/g de pienso, que confirma el efecto inhibidor que *B.toyoi* ejerce sobre las cepas de *E.coli* enterotóxicas en lechones y terneros. Estos resultados muestran que la administración de productos bacterianos viables puede ser una forma efectiva de prevenir la disentería del conejo.