THE MODE OF ACTION OF ANTIBIOTICS IN THE NUTRITION OF THE DAIRY CALF. I. EFFECT OF TERRAMYCIN ADMINISTERED ORALLY ON THE PERFORMANCE AND INTESTINAL FLORA OF YOUNG DAIRY CALVES

J. J. RADISSON, C. K. SMITH, AND G. M. WARD

Departments of Dairy and Microbiology and Public Health
Michigan State University, East Lansing

The need for information on the mechanism(s) involved in the effect of certain antibiotics in stimulating the appetite of nonruminating calves and in causing certain benefits to the animal led to this investigation. Fifteen 3-day-old calves were kept on the experiment for 9 weeks. The results suggest that the presence of enteric streptococci limits the growth potential of young animals. The need for further study to develop improved techniques for determining the extent to which antibiotics affect the intestinal flora of animals is indicated. Editor.

It is now well established (1, 13) that the feeding of certain antibiotics, such as aureomycin and terramycin, to young nonruminating calves stimulates appetite, increases growth rate, reduces intestinal disturbances, and improves physical appearance. However, there is still a lack of agreement as to the actual mechanism(s) involved. Most of the hypotheses have been challenged by various workers who have been unable to correlate growth stimulation with changes in the intestinal flora.

In view of these divergences of reports and of the paucity of information regarding the effects of antibiotics on the intestinal flora of dairy calves, an experiment was initiated in order to investigate the effect of orally administered terramycin on representative groups of the intestinal flora of dairy calves in an attempt to correlate changes, if any, with the performance of the animals.

This paper is the first of a series of publications on the mode of action of antibiotics in the nutrition of the dairy calf. It is primarily intended to show some of the limitations of standard bacteriological techniques for a study of the mode of action of antibiotics on the flora of the digestive tract of animals.

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3 Present address: c/o DUQUESNE S.A., Montfort (Eure), France.

4 Present address: Kansas State College, Manhattan.
MATERIAL AND METHODS

Animals used, feeding and management. Fifteen 3-day-old dairy calves were kept on experiment for 9 weeks. The 10 control animals received a ration consisting of whole milk in proportion to body weight and hay and grain fed according to appetite. The five animals in the experimental group received the same basal diet, plus a terramycin supplement$^5$ added to the milk so as to provide 1 mg. of terramycin per pound of body weight daily.

Bacteriological methods. Fecal samples were collected twice a week directly from the rectum of the calves into sterile Petri dishes. The microscopic method developed by Bortree et al. (2) was used to determine the total bacterial count. Lauryl tryptose broth (10) was chosen as a presumptive medium to estimate the number of coliforms present. Dextrose azide broth (12) was used as an enrichment medium for streptococci; the positive tubes were confirmed by transfer to ethyl violet azide broth (11).

Bacterial sensitivity to antibiotics was determined as follows: single colonies of coliform bacteria were isolated on eosin methylene blue agar plates. Isolation of single colonies of enteric streptococci was performed according to the general procedure established by Sherman (16); in this procedure, the reaction in litmus milk was considered as a test per se and was not used in the identification of the types of streptococci isolated. The standard paper disk method was used for the assays.

During the process of isolation of enteric streptococci for sensitivity assays, some indications were obtained that the metabolism of some of the bacteria living in the intestinal tract of the calves might be affected by feeding terramycin. In order to test this hypothesis, the fermentative activity in sterile skimmilk of several strains of streptococci isolated from the feces of control and terramycin-fed calves was investigated. The strains isolated from the feces of control calves were inoculated each into eight series of 10 test tubes containing 9 ml. of skimmilk and 0 to 50 $\gamma$ of terramycin per milliliter. The cultures were incubated at 35$^\circ$ C. One tube of each concentration was taken out of the incubator every 6 hours for 48 hours and after 96 hours, and the pH of the skimmilk was determined with a Cenco pH meter. After 24 hours of incubation, one tube of each series was transferred into 10 tubes of skimmilk free from terramycin. A second transfer was made similarly with the 24-hour cultures of the first transfer as the inocula, and pH measurements were taken as previously described. The strains of enteric streptococci isolated from the feces of terramycin-fed calves were inoculated each into a series of 10 tubes of skimmilk free from terramycin. Two successive transfers were also made, and pH measurements were taken according to the procedure and schedule previously described.

Excretion of terramycin. Fecal samples were collected at 0, 6, 9, 12, 15, and 24 hours after the supplement was fed for the first time. The concentration and total amount of terramycin excreted in the urine and in the feces as functions of the intake were also determined. Feces and urine were collected quantitatively.

$^5$ Bi-Con TM-5, guaranteed to contain 5 g. of pure terramycin hydrochloride per pound, was supplied by Charles Pfizer and Co. Inc., Brooklyn 6, N. Y.
every 6 hours for 24 hours at 13 days of age and every 6 hours for 48 hours at
19, 32, and 64 days of age. A modification of the standard paper disk method
was used for the assays.

RESULTS

Effects of terramycin on growth rate and feed efficiency. Terramycin feeding
significantly increased ($P < 0.01$) the rate of weight gain by 33% above that of
the control animals. The stimulation of growth was greatest during the first
6 weeks. The growth of the control calves was depressed during the first 3
weeks, but their average gain for the first 9 weeks of age was 16% above the
Ragsdale standard. The incidence of scours was low and was not affected by
the treatment. Appetite and feed efficiency of the terramycin-fed calves were
significantly increased above those of the controls. Both appetite and feed effi-
ciency were significantly correlated with the rate of gain.

Bacteriological findings. The results of the total bacterial counts and the
number of coliforms and enteric streptococci in the feces of the calves are pre-
sented graphically in Figure 1. The total and coliform counts were not signifi-
cantly different in the control and in the terramycin-fed group. The numbers
of the enteric streptococci averaged 162,000 per gram of wet fecal material as

Fig. 1. Effect of feeding terramycin from 3 to 66 days of age on the total bacterial count
and the number of coliforms and enteric streptococci present in the feces of dairy calves.
TABLE 1
Effects of terramycin administered orally on the sensitivity to terramycin of strains of coliform bacteria isolated from the feces of young dairy calves

<table>
<thead>
<tr>
<th>Treatment of the calves</th>
<th>No. of samples</th>
<th>No. of strains</th>
<th>No. of strains resistant to various levels of terramycin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>13</td>
<td>51</td>
<td>9 10 14</td>
</tr>
<tr>
<td>Terramycin</td>
<td>21</td>
<td>65</td>
<td>82 65 65</td>
</tr>
</tbody>
</table>

TABLE 2
Effects of terramycin administered orally on the sensitivity to terramycin of strains of enteric streptococci isolated from the feces of young dairy calves

<table>
<thead>
<tr>
<th>Treatment of the calves</th>
<th>No. of samples</th>
<th>No. of strains</th>
<th>No. of strains resistant to various levels of terramycin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>15</td>
<td>62</td>
<td>8 13 16</td>
</tr>
<tr>
<td>Terramycin</td>
<td>31</td>
<td>117</td>
<td>60 80 85</td>
</tr>
</tbody>
</table>

compared to 1.44 million per gram in the control group. This difference was found to be significant at the 1% level of probability (the logarithmic averages were used in the statistical analysis). The average enteric streptococci counts for each calf were negatively correlated ($r = -0.63$, significant at the 1% level of probability) with the rate of growth of the animals evaluated in comparison with the Ragsdale standard.

Data pertaining to the studies on sensitivity of coliforms and enteric streptococci to terramycin are summarized in Tables 1 and 2, respectively. With both groups of bacteria, there was a marked increase in the numbers of strains resistant to terramycin when this antibiotic was fed. The values obtained for sensitivity to aureomycin were similar to those obtained with terramycin and therefore are not presented. Coliforms appeared rather sensitive and enteric streptococci highly resistant to dihydrostreptomycin independently of their sensitivity to terramycin and whether or not they were isolated from control or terramycin-fed calves (data not presented).

All but three of the strains of streptococci isolated appeared to be true enterococci, as evidenced by their ability to produce turbidity in brain-heart infusion broth and in tryptose phosphate with 6.5% salt, and to reduce litmus milk. However, the strains isolated from terramycin-fed calves were characterized by a much lower ability to curdle litmus milk, as compared to strains isolated from control calves.

Figure 2 (A and B) illustrates the results obtained in the study concerning the influence of terramycin on cultures of enteric streptococci in skimmilk. Terramycin added to the skimmilk produced a significant decrease in the activity of the strains isolated from the control calves, as measured by acid production. Strains isolated from the feces of terramycin-fed calves followed closely the pattern observed when 50 $\gamma$ of terramycin per milliliter were added to strains isolated from control calves. No important changes in bacterial activity were
observed in the first transfer in skimmilk free of the antibiotics, as compared with the results of the initial inoculation. After the second transfer, the influence of the initial addition of terramycin was no longer apparent.

**Excretion of terramycin in the feces and the urine.** Terramycin was found in the feces 6 to 9 hours after the first administration. The concentrations increased in the following collections. The values obtained after 24 hours ranged from 57 to 250 \( \gamma \) of terramycin per gram of feces on a dry matter basis. The average concentrations of terramycin found in the feces at 13, 19, 32, and 64 days of age were 343, 756, 300, and 121 \( \gamma \) per gram of dry fecal material, respectively. Similarly, the percentages of terramycin intakes excreted were 33, 21, 26, and 21 in the feces and 12, 9, 12, and 4 in the urine at 13, 19, 32, and 64 days of age, respectively. The concentrations of terramycin in the urine varied from 0.7 to 5.2 \( \gamma \) per milliliter.

**DISCUSSION**

Since the maximum growth response to terramycin in this experiment was obtained during the first few weeks, when the growth rate of the control calves seemed to be depressed, it would appear that terramycin relieved a growth...
depression rather than produced a true stimulation of growth. This is in agreement with the hypothesis proposed by Coates et al. (3). However, it should be noted that in the present experiment the incidence of scours was low and was not affected by the treatment. Furthermore, the number of coliform organisms, which is considered among the main causative agents of scours, was not influenced by terramycin feeding.

The results of this investigation would suggest that the presence of enteric streptococci possibly limited the growth potential of the animals since there was a negative correlation between the number of these bacteria in the feces and the growth rate of the calves. If so, part of the growth increase obtained with terramycin in this experiment may have been due to the effect of this antibiotic on enteric streptococci. In a similar and concurrent experiment (8), aureomycin was fed to young dairy calves kept in the same environment as the animals used in the present study. Growth was stimulated approximately to the same extent by aureomycin as by terramycin, but the effect of aureomycin on streptococci was less evident. Voelker and Cason (18) and Rusoff et al. (15) found no consistent differences in the intestinal flora of dairy calves when fed terramycin or aureomycin.

Similar inconsistencies abound in the literature pertaining to the effect of antibiotics on the intestinal flora of animals. In practically all of these studies, only changes in number of various groups of bacteria have been considered. It is evident that this is only part of the picture; bacterial counts merely indicate an approximation of the number of bacteria in the intestine and give no indication whatsoever concerning their activity. Since terramycin is known to be a bacteriostatic rather than a bactericidal agent when used at low concentrations, the number of the various groups of microorganisms in the feces may not be markedly affected, whereas their growth rate and metabolism may be significantly reduced in some cases or enhanced in others. The effect of terramycin on the ability of enteric streptococci to ferment litmus milk and skimmilk is an example of such changes in the physiology of intestinal bacteria. Of greater significance is the increased resistance of intestinal bacteria to antibiotics after antibiotic feeding; this was demonstrated in the present experiment and has also been reported by others (4, 5, 6, 7, 9, 14, 17). The significance of this increased resistance of bacteria to antibiotics, in relation to the sensitivity of these bacteria to the host defense mechanisms, has been studied and will be presented later.

**SUMMARY**

An experiment was conducted to investigate the effects of feeding terramycin on the performance and intestinal microflora of dairy calves from 3 days to 9 weeks of age. Terramycin administration increased significantly the rate of weight gain by 33% above that of the control animals. Appetite and feed efficiency were significantly increased. The incidence of scours was low and was not affected by the treatment. Terramycin was detected in the feces 6 to 9 hours after the first feeding. High concentrations of terramycin were found in the
feces during continuous feeding of terramycin and the antibiotic was also present in the urine.

Bacteriological studies revealed no marked difference in total and coliform counts attributable to the feeding of terramycin. However, there was a significant decrease in the number of enteric streptococci when terramycin was administered. Antibiotic sensitivity assays indicated an increase in the number of resistant strains of coliforms and enteric streptococci in the feces of calves fed terramycin. The normal metabolism of enteric streptococci appeared to be impaired by the presence of terramycin in vivo and in vitro. These bacteriological studies indicated that improved techniques should be used to determine the extent to which antibiotics affect the intestinal flora of animals.

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REFERENCES


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