Growth of Mice Fed Milk Fermented with \textit{Lactobacillus acidophilus}\textsuperscript{1}

KATHARINE K. GRUNEWALD* and LAURA K. MITCHELL

Department of Foods and Nutrition, Kansas State University, Manhattan, Kansas 66506

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ABSTRACT

The effects of milk fermented by \textit{Lactobacillus acidophilus} on growth of Swiss albino mice were tested. Mice were fed a stock diet and drinking water containing one of these milk additions: no supplementary milk (control), 10\% liquid skim milk, 10\% milk containing a viable culture of \textit{L. acidophilus}, or 10\% milk fermented by \textit{L. acidophilus}. After 4 weeks mice receiving the fermented acidophilus milk had consumed more total feed and gained more weight than had the control mice, but not more than those fed the liquid skim milk or the nonfermented acidophilus milk.

Fermented foods have been important from a historical standpoint not only for their improved keeping qualities, but also for their nutritional value. Although it is generally believed that the nutrient content of a fermented food basically reflects that of the food from which it is made, there is some evidence for microbial synthesis of B-complex vitamins during the fermentation process (12,18). Harmon and Alford (8) suggested that the fermentation process might improve bioavailability of milk protein. In one study, infants fed kefir, a fermented milk, retained more nitrogen, calcium, and iron than did those fed fresh milk (24).

In the experiment reported here mice fed a diet supplemented with fermented milk exhibited greater weight gains than those receiving no supplementation. The microorganism selected to ferment the milk was a \textit{Lactobacillus acidophilus} strain isolated from the feces of mice.

MATERIALS AND METHODS

Animals

One hundred healthy, male weanling mice (weighing 10 ± 2 g) were obtained from our departmental breeding colony of random-bred Swiss albino mice. Animals were assigned to four treatment groups (25 to a group) on a littermate basis so that each group would be similar genetically. Throughout the 28-d trial, the mice were housed in polypropylene cages in a temperature-controlled room (22°C) with a 12-h light-dark cycle. They were allowed free access to a stock diet (Rodent Laboratory Chow, Ralston Purina Co., St. Louis, MO) and deionized drinking water containing: no supplementary milk (control), 10\% liquid skim milk, 10\% nonfermented acidophilus milk or 10\% fermented acidophilus milk.

Each treatment group contained 25 mice, which were each identified by ear punch procedures and housed five mice per cage. One apparently healthy mouse receiving the fermented milk died during the first week of the study. Our results do not include data from that animal.

| TABLE 1. Characteristics of the \textit{Lactobacillus acidophilus} strain. |
|------------------|------------------|------------------|
| Characteristic   | Reaction         | Characteristic   | Reaction         |
| Growth at 15°C   | –                | Mannitol         | –                |
| Growth at 45°C   | +                | Mannose          | +                |
| Catalase         | –                | Melezitose       | –                |
| Amygdalin        | +                | Melibiose        | –                |
| Arabinose        | –                | Raffinose        | –                |
| Cellobiose       | +                | Rhamnose         | –                |
| Fructose         | +                | Ribose           | –                |
| Galactose        | +                | Salicin          | +                |
| Glucose (acid)   | –                | Sorbitol         | –                |
| Glucose (gas)    | –                | Sucrose          | +                |
| Glucuronate      | –                | Trehalose        | –                |
| Lactose          | +                | Xylose           | –                |
| Maltose          | +                | Esculin          | +                |

\textsuperscript{1}Reference to a company and/or product is only for purposes of information and does not imply approval or recommendation of the product over others not mentioned.

Preparation of supplementary milks

Powdered skim milk (Difco Laboratories, Detroit, MI) was used for all supplementary milk treatments. Milk was reconstituted (100 g/L) and autoclaved at 118°C for 15 min in 100-mL portions, then stored at room temperature until cultured or fed.
TABLE 2. Growth responses to Lactobacillus acidophilus fermented and nonfermented milks.

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Average wt. gain (g/28 days)</th>
<th>Feed intake (g/28 days)</th>
<th>Feed efficiency</th>
<th>Femur wt. (mg)</th>
<th>Epididymal fat pad wt. (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>17.6 ± 5 ab</td>
<td>Stock diet: 93.9 ± 1.6 a</td>
<td>93.9 ± 1.6 a</td>
<td>42.9 ± 0.7 a</td>
<td>0.25 ± 0.1 a</td>
</tr>
<tr>
<td>Water + 10% milk</td>
<td>18.1 ± 4 ab</td>
<td>Milk solids: 96.5 ± 1.3 a</td>
<td>99.1 ± 1.4 ab</td>
<td>44.0 ± 0.9 ab</td>
<td>0.25 ± 0.1 ab</td>
</tr>
<tr>
<td>Water + 10% nonfermented acidophilus milk</td>
<td>18.3 ± 3 ab</td>
<td>Total solids: 2.6 ± 0.7 a</td>
<td>97.2 ± 2.2 ab</td>
<td>43.8 ± 1.2 a</td>
<td>0.29 ± 0.2 b</td>
</tr>
<tr>
<td>Water + 10% fermented acidophilus milk</td>
<td>19.0 ± 3 b</td>
<td></td>
<td></td>
<td>45.0 ± 0.8 a</td>
<td>0.28 ± 0.1 b</td>
</tr>
</tbody>
</table>

1Mean ± SEM of 20 to 25 mice.
abMeans within columns not bearing common superscripts differ, P<.05.

Feathered milk initially was prepared by inoculating liquid skim milk with a viable culture of L. acidophilus in lactobacillus MRS broth (Difco Laboratories) and incubating it at 37°C for 24 h. Starter viability was maintained in fermented milk fed to mice by daily successive 5% inoculations and incubation at 37°C for 24 h before feeding. Milk prepared in that manner had an average pH of 4.8 and an average viable count of 7 × 10⁸ per ml, as enumerated weekly in MRS agar (MRS broth + 1.5% agar) after incubating in a gaspuck H₂CO₃ system (BBL, Cockeysville, MD) for 48 h.

A nonfermented acidophilus milk was also prepared. L. acidophilus was cultured in 10-ml portions of MRS broth at 37°C for 24 h. Bacterial cells, harvested by centrifugation at 2500 × g for 3 min, were washed with sterile saline solution and added to 10 ml of milk. After being well mixed, this nonfermented acidophilus milk, having an average viable count of 1 × 10⁶ per ml, was fed to the animals.

Feeding and sacrifice procedures

All supplementary milks were given fresh daily at a 10% level (v/v) in the drinking water in clean water bottles. Observing that our animals consumed most of their diet during the dark cycle, we gave the milk treatments immediately before this time to maximize consumption of a fresh product. Consumption of liquids was estimated by daily measuring disappearance. Consumption of stock diet was measured by weighing feed on an air-equilibrated basis. Feed efficiencies were determined from total weight gains and combined consumption of stock diet and milk solids. After 4 weeks animals were sacrificed and weighed, and their epididymal cells, harvested by centrifugation at 2500 × g for 3 min, were washed with sterile saline solution and added to 10 ml of milk. After being well mixed, this nonfermented acidophilus milk, having an average viable count of 1 × 10⁶ per ml, was fed to the animals.

RESULTS

Weight gains of mice during the 28-d trial are given in Table 2. Mice fed the fermented acidophilus milk gained an average of 8% more weight (P<0.05) than did the control mice, but not significantly more than those fed the other milks. Average weight gains of mice fed the liquid skim milk or the nonfermented acidophilus milk were numerically intermediate between those of the control and fermented-milk-fed mice but were not significantly different from them.

Epididymal fat pad weights of mice fed either fermented or nonfermented acidophilus milks were heavier (P<0.05) than those of the control mice. Femur weights were not significantly different among treatment groups.

The fermented-milk-fed mice consumed an average 8% more total dietary solids (P<0.05) than did the control mice, but not significantly more than the mice fed the liquid skim milk or the nonfermented acidophilus milk. We observed an average disappearance of about 0.8 ml of milk (8-ml liquid) for each of the animals consuming the supplementary milks. Feed efficiencies were not significantly different among treatment groups.

DISCUSSION

In our study, growth responses of mice receiving a stock diet plus either drinking water, skim milk, nonfermented acidophilus milk or fermented acidophilus milk, were compared. The finding that fermented-milk-fed mice consumed more feed and gained more weight than water-fed mice is noteworthy in that all animals had free access to their respective diets and presumably ate enough to satisfy their hunger. Yet mice receiving the fermented milk had a greater voluntary consumption of total solids than non-supplemented mice. The greater feed consumption by the fermented-milk-fed mice could not be attributed solely to consumption of supplementary milk solids, but rather to a combination of stock diet and milk solids.

Although the reason for the greater feed consumption by the fermented-milk-fed mice is not clear, one might speculate that those effects were attributed to milk nutrients or possible microbial factors in the fermented milk. L. acidophilus produces antibiotics (11,19,20); and antibiotics enhance weight gain and feed consumption in growing animals (1,10).

However, our data indicate that the observed effects were probably not exclusively due to microbial factors. Mice consuming the fermented milk, which contained milk nutrients and microbial factors, did not consume more feed or gain more weight than mice consuming the non-inoculated skim milk containing milk solids only. So our data suggest that fermented milk does not offer an advantage over skim milk when growth responses are measured. The fermented-milk-fed mice did, however, consume more total solids than the mice receiving unsupplemented water.

We also weighed epididymal fat pads and femurs of our mice to obtain additional measures of growth. The fermented-milk-fed mice had heavier epididymal fat pads than control mice, suggesting that this store of body fat is greater in fermented-milk-fed mice. Femurs were not signific-
cly heavier in fermented-milk-fed mice than water-fed mice.

A nonfermented acidophilus milk was also fed to mice. Feed intake and weight gain of those animals were not significantly greater than those of water-fed mice, but the differences approached significance. Mice receiving the nonfermented acidophilus milk had significantly heavier epididymal fat pads than water-fed mice reinforcing the trends towards greater weight gain by these animals. Even though this milk was not fermented, it is possible that some fermentation could have occurred in the water bottles during feeding. Although the extent of this process is not known, we tried to minimize this possibility by providing mixtures fresh daily before the dark cycle when mice consumed most of their feed.

Others have investigated the effects of fermented milks on growth. Hargrove and Alford (8) found that rats fed yogurt, milk fermented by Lactobacillus bulgaricus and Streptococcus thermophilus, gained more weight than those fed milk. Simhaee and Keshavarz (21) found yogurt proteins superior to those of skim milk for growing chicks. Results from our study may differ with those of others because of differences in species and strain of microorganism used or of differences in dietary intake and composition. Milk solids in our study comprised a small fraction (about 3%) of the total diet for the mice fed the supplementary milk; and it is possible that the differences among treatment groups would have been more marked if the milk supplements had occupied a larger portion of the diet. On the other hand, milk, if given as the sole source of nutrients, will not provide adequate amounts of several minerals for optimal rodent growth (14).

L. acidophilus is a normal inhabitant in the gastrointestinal tracts of humans and other warm-blooded mammals (22,23). Several roles have been suggested for this microbially species, including antibiotic production (19,20), bile salt deconjugation (6), competition for available niches in the gut (4) and production of lactic acid, which inhibits many undesirable bacteria (2). The interrelationships between lactobacilli and host health have been examined in several reviews published by the National Dairy Council (15-17).

We used a microbial strain isolated from the feces of our mice for the acidophilus milks because there is some evidence that various strains of L. acidophilus are host specific (5,13). The microorganism thus obtained was believed to be compatible with the gastrointestinal tracts of our mice.

There is increasing interest in the nutritional value of fermented milks. Our study shows that mice receiving fermented acidophilus milk consumed more feed and gained more weight than mice receiving no supplementation; but not more than those receiving supplementary skim milk. Further research is needed to clarify the effects of various fermented milks and their nutritional value.

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REFERENCES