Utilization by the Rat of Nitrogen, Calcium, Phosphorus, and Magnesium in Sterile Concentrated Milk Stabilized with Polyphosphates

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Abstract

The effects of processing and addition of polyphosphates upon the nutritive value of ultra high-temperature short-time sterilized concentrated milks were investigated. Diets containing 9% protein from spray-dried whole milk, or from sterile concentrated milks with and without 0.2% polyphosphate additives, were fed in growth restricting amounts to weanling rats in a reversal experiment. Food intake, fecal and urinary loss were determined for nitrogen, phosphorus, calcium, and magnesium. Analysis of variance indicated that no significant difference in protein quality exists between the various milks tested as assessed by biological value or by true and apparent digestibility of nitrogen. The retention of phosphorus and calcium was not appreciably affected by addition of polyphosphates to sterile milk concentrates, but the apparent absorption of both minerals was significantly higher. Magnesium from the spray-dried whole milk diet was absorbed to a significantly greater extent than was magnesium from only one of the ultra high-temperature short-time sterile concentrated milk diets containing polyphosphates. It is possible to conclude that young growing rats are able to utilize the phosphorus, calcium, magnesium, and proteins of sterile concentrated milks containing polyphosphates as well as do rats fed spray-dried whole milk or concentrated milk without added polyphosphates.

Concentrated whole milk products sterilized by the ultra high-temperature, short-time method (UHTST) and stabilized by the addition of polyphosphates to prevent gelation have been developed by several laboratories (9, 22). Sterile concentrated milks have high flavor acceptability (12) and possess several unique properties in food preparation (13). As it is a potentially important milk product, it was considered worthwhile to study the nutritive value of UHTST sterile milk concentrates with and without added polyphosphates.

The changes which can occur in the nutritive value of milk proteins as a result of processing have been reviewed by Porter (15, 16) and by Bender (2). The effects of heat upon the nutritive value of protein, and the factors which influence the changes which occur in proteins in heat-processed foods, also have been discussed in a series of articles by Danehy and Pigmen (4), Rice and Benk (18), and Reynolds (17). When changes are severe enough, the nutritive value of the protein is lowered. The amino acid most susceptible to heat damage is lysine, which has a tendency to interact with carbohydrate to form a complex not easily split by digestive enzymes.

Since the proteins of sterile concentrated milks are exposed to heat for only a short time, one would expect little lowering of their nutritive value; however, the high lactose concentration and greater protein instability of these milks may produce unforeseen effects.

Sodium polyphosphates are used extensively by the food industry (20), but their metabolism is not understood (8). One study which indicates that rats can successfully absorb and utilize phosphorus from several sodium polyphosphates and hexametaphosphate is that by Schreier and Noller (19).

Protein quality was assessed by determining biological value and digestibility of nitrogen for the weanling rat on a restricted growth regime. Since a nutritionally important interrelationship exists between phosphorus, calcium, and magnesium (7), the balance and apparent absorption of these minerals were determined to establish what effect, if any, polyphosphate additive and processing have upon the availability of these minerals.

Experimental Procedure

The UHTST concentrated milks used in this experiment were manufactured by the Dairy
Phosphates being added before sterilization of milk containing 0.2% Na tetrametaphosphate; whole milk was used as a control. The canned concentrates were stored at 4°C until use four months later. Spray-dried whole milk was used as a control.

Diet SQ, UHTST milk containing 0.2% SQ polyphosphate; Diet RI, spray-dried whole milk. Based on previous analyses of the milks, phosphorus was brought to a level of 0.45-0.57% of the diet by adding Ca₃(PO₄)₂. Calcium content of the diets varied from 0.48-0.55%, magnesium from 0.043-0.052%, and nitrogen from 1.46-1.52%. Trace minerals made up 0.728% of each diet, vitamin mix made up 0.500%, and Cerelose the difference, approximately 64-68%. Note that the vitamin mix contains 5.0 µg per gram of diet of DL-methionine. By supplementing with sulfur-containing amino acids, which are limiting for the nutritive value of total milk proteins, small changes in lysine availability should result in a greater decrease in biological value than normally would be apparent.

Thirty male Sprague-Dawley weanling rats, averaging 45-48 g body weight, were housed individually in stainless steel metabolism cages. Each group of six rats was assigned to one of the diets for two weeks (Period 1); at the end of this time each group was randomly switched to another diet for an additional two weeks (Period 2), with the restrictions that the groups receiving control diets (D or C) now receive experimental diets and that no group receives the same diet as in Period 1. Food was restricted to 11.4-12.1 g per day during Period 1, and 12.2-12.8 g per day during Period 2. Deionized water was provided ad libitum. Urine and feces were collected separately during the second week of each period.

Results and Discussion

Weight and growth rates. There were no significant differences between groups for weight at the beginning of the experiment or weight at the beginning or end of collection Periods 1 or 2. Average weights varied from 76.5 ± 2.0 to 84.3 ± 1.4 g during Period 1 and from 128.1 ± 3.3 to 139.0 ± 1.5 g during Period 2; these differences were not significant. The rats of Group 5 showed consistently lower gain in weight per day during both periods (P < 5%, Table 1), reflecting a slightly lower food intake.

Nitrogen utilization. Data for the mean biological value, nitrogen balance, and true and apparent digestibility of nitrogen in the experimental diets are presented in Table 1. Although nitrogen balance is significantly lower for rats in Groups 3 and 5, fed Diets T and D, respectively (P < 5%), this pattern does not appear in Period 2 after the diets have been changed. No significant difference is seen in biological value or in true or apparent digestibility of nitrogen in any of the milks studied. The lower biological value and higher apparent and true digestibility in Period 2 as compared to Period 1 are functions of the age of the animal and have been observed by other workers (14, 21).

The data obtained for nitrogen show that the

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Footnotes:

1. Percentage based on 36.5% total solids of concentrated milk.
2. Supplied by the Dean Milk Company, Campaign, Illinois.
3. Trade name for a product of the Monsanto Company, St. Louis.
4. Composition of mix in grams: NaCl, 7.690; Na₂CO₃, 10.060; K₂CO₃, 15.340; MgCO₃, 10.420; ZnCO₃, 0.884; FeSO₄·7H₂O, 1.240; CuSO₄·5H₂O, 0.196; MnSO₄·H₂O, 1.540; KI, 0.004.
5. Amount per gram of diet: Vitamin A, 1.5 I.U.; vitamin D, 0.6 I.U.; thiamine hydrochloride, 4.0 µg; riboflavin, 4.0 µg; Ca pantothenate, 4.0 µg; niacin, 10.0 µg; pyridoxine hydrochloride, 1.0 µg; menadione, 0.4 µg; folic acid, 0.2 µg; biotin, 0.1 µg; vitamin B₁₂, 0.1 µg; and L-methionine, 5.0 mg. All animals received two drops of cod-liver oil (Parke Davis Company, Detroit) every week.

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TABLE 1. Weight gain, nitrogen balance, apparent and true digestibility, and biological value of nitrogen for rats fed milk and milk with added phosphates

<table>
<thead>
<tr>
<th>Group</th>
<th>Diet</th>
<th>Gain per day (avg)</th>
<th>Nitrogen balance</th>
<th>Digestibility of milk nitrogen</th>
<th>Biological value&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(g) (P)</td>
<td>(mg/day) (P)</td>
<td>Apparent&lt;sup&gt;b&lt;/sup&gt; (%)</td>
<td>True&lt;sup&gt;c&lt;/sup&gt; (%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5%</td>
<td>5%</td>
<td>5%</td>
<td>5%</td>
</tr>
<tr>
<td>Period 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>C</td>
<td>4.2 *±0.14&lt;sup&gt;d&lt;/sup&gt;</td>
<td>109.2±3.3</td>
<td>87.0±0.47</td>
<td>95.2±0.47</td>
</tr>
<tr>
<td>2</td>
<td>H</td>
<td>4.3 *±0.15</td>
<td>118.7±1.5</td>
<td>88.4±0.48</td>
<td>96.3±0.49</td>
</tr>
<tr>
<td>3</td>
<td>T</td>
<td>3.7 *±0.25</td>
<td>106.1±4.4</td>
<td>87.5±0.83</td>
<td>95.7±0.84</td>
</tr>
<tr>
<td>4</td>
<td>SQ</td>
<td>3.8 ±0.21</td>
<td>106.7±5.4</td>
<td>87.7±1.04</td>
<td>95.7±1.06</td>
</tr>
<tr>
<td>5</td>
<td>D</td>
<td>3.5 *±0.16</td>
<td>98.4±3.8</td>
<td>85.4±1.29</td>
<td>93.4±1.28</td>
</tr>
<tr>
<td>Period 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>T</td>
<td>3.4 ±0.10</td>
<td>111.8±2.6</td>
<td>90.2±0.37</td>
<td>98.3±0.37</td>
</tr>
<tr>
<td>2</td>
<td>D</td>
<td>3.4 ±0.22</td>
<td>113.7±2.9</td>
<td>90.8±0.66</td>
<td>98.5±0.57</td>
</tr>
<tr>
<td>3</td>
<td>C</td>
<td>4.1 *±0.27</td>
<td>120.1±3.5</td>
<td>90.2±0.72</td>
<td>98.2±0.64</td>
</tr>
<tr>
<td>4</td>
<td>H</td>
<td>3.9 ±0.14</td>
<td>118.5±2.9</td>
<td>91.4±0.68</td>
<td>99.0±0.57</td>
</tr>
<tr>
<td>5</td>
<td>SQ</td>
<td>3.2 *±0.22</td>
<td>107.7±3.6</td>
<td>91.5±0.54</td>
<td>99.1±0.39</td>
</tr>
</tbody>
</table>

<sup>a</sup> Values in the same category with common superscripts are not significantly different by Duncan's Multiple Range test. Values with no superscripts are not significantly different from either group.

<sup>b</sup> % Apparent digestibility = \( \frac{\text{total N intake} - \text{total fecal N}}{\text{total N intake}} \times 100 \).

<sup>c</sup> % True digestibility = \( \frac{\text{total N intake} - (\text{total fecal N} - \text{metabolic fecal N})}{\text{total N intake}} \times 100 \).

<sup>d</sup> Biological value = 100 * \( \frac{\text{N intake} - (\text{total fecal N} - \text{metabolic fecal N}) - (\text{total urinary N} - \text{endogenous urinary N})}{\text{N intake} - (\text{total fecal N} - \text{metabolic fecal N})} \).

<sup>x</sup> Weight in grams of dry matter consumed × 1.2 mg = mg/day. (Forbes, personal communication.)

<sup>y</sup> (EUN) = 0.60 × (body weight)\( ^{0.65} \). (Forbes, personal communication.)

<sup>z</sup> Standard error of the mean.

The nutritive value for growing rats of the protein of UHTST sterilized concentrated milk and of milk containing polyphosphates is similar to the nutritive value of spray-dried milk. The possibility that the biological value of UHTST milk stored at higher temperature may be adversely affected on prolonged storage is not precluded.

**Mineral utilization.** The data for phosphorus, calcium, and magnesium balance and apparent absorption are given in Table 2. Although phosphorus balance is significantly higher during Period 1 for rats fed sterilized concentrated milk containing polyphosphates than for rats fed the sterilized concentrated control, the data for Period 2 do not confirm this finding. Apparent absorption of phosphorus is significantly lower in Period 1 (P < 1%) and in Period 2 (P < 5%) for rats fed sterilized concentrated milk without added phosphates as compared to rats fed sterilized milk with added phosphates. There are no significant differences seen in calcium balance for both feeding periods. The apparent absorption of calcium is significantly higher for rats fed one of the phosphate-containing milks (SQ, P < 1%) than for rats fed the control milk, and is significantly higher in Period 2 for rats fed two of the phosphate-containing milks (SQ, H; P < 1%) than for rats fed the control milk. The mean apparent absorption of both calcium and phosphorus for rats fed sterile concentrated milks containing polyphosphates was not significantly different during either period from the mean apparent absorption of calcium or phosphorus for rats fed dried whole milk.

While appreciable positive balances, with only one exception (magnesium balance for rats on Diet T, Period 2), were obtained for phosphorus, calcium, and magnesium, they are generally lower than those reported by Forbes (7) for rats of similar age and gaining at comparable rates of growth, about 3-4 g per day. The diets used by Forbes were not strictly comparable to those used in the present study, since they contained about the same amounts of phosphorus and magnesium, whereas the calcium content was 0.8% and the protein content 12.5%.

Comparisons of the data for magnesium show that the mean differences for balance and apparent absorption between the same diets fed
Table 2. Balance and apparent absorption of phosphorus, calcium, and magnesium in milk and milk with added phosphates

<table>
<thead>
<tr>
<th>Group</th>
<th>Diet</th>
<th>Balance P (mg/day)</th>
<th>Apparent absorption P (%)</th>
<th>Balance Ca (mg/day)</th>
<th>Apparent absorption Ca (%)</th>
<th>Balance Mg (mg/day)</th>
<th>Apparent absorption Mg (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Period 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>C</td>
<td>26.8 ±1.4</td>
<td>&lt;1%</td>
<td>65.2 ±3.3</td>
<td>31.8 ±0.5</td>
<td>57.6 ±1.0</td>
<td>0.71 ±0.02</td>
</tr>
<tr>
<td>2</td>
<td>H</td>
<td>33.9 ±0.7</td>
<td>&lt;1%</td>
<td>76.3 ±1.3</td>
<td>33.8 ±0.7</td>
<td>65.1 ±1.6</td>
<td>1.23 ±0.23</td>
</tr>
<tr>
<td>3</td>
<td>T</td>
<td>32.2 ±1.3</td>
<td>&lt;1%</td>
<td>76.6 ±1.5</td>
<td>30.4 ±1.1</td>
<td>64.2 ±1.8</td>
<td>1.16 ±0.07</td>
</tr>
<tr>
<td>4</td>
<td>SQ</td>
<td>32.8 ±1.6</td>
<td>&lt;5%</td>
<td>78.9 ±3.7</td>
<td>30.7 ±1.3</td>
<td>67.4 ±2.7</td>
<td>1.17 ±0.09</td>
</tr>
<tr>
<td>5</td>
<td>D</td>
<td>30.6 ±1.1</td>
<td>&lt;1%</td>
<td>81.1 ±1.1</td>
<td>32.7 ±0.6</td>
<td>71.8 ±1.8</td>
<td>0.73 ±0.07</td>
</tr>
<tr>
<td>Period 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>T</td>
<td>29.0 ±1.4</td>
<td>&lt;5%</td>
<td>76.4 ±1.2</td>
<td>35.1 ±0.6</td>
<td>62.7 ±1.4</td>
<td>-0.34 ±0.67</td>
</tr>
<tr>
<td>2</td>
<td>D</td>
<td>30.8 ±0.9</td>
<td>&lt;1%</td>
<td>80.6 ±1.5</td>
<td>39.7 ±1.1</td>
<td>69.2 ±2.2</td>
<td>0.82 ±0.08</td>
</tr>
<tr>
<td>3</td>
<td>C</td>
<td>27.9 ±1.7</td>
<td>&lt;1%</td>
<td>71.2 ±2.7</td>
<td>36.9 ±2.2</td>
<td>57.6 ±2.8</td>
<td>0.56 ±0.19</td>
</tr>
<tr>
<td>4</td>
<td>H</td>
<td>30.3 ±1.4</td>
<td>&lt;1%</td>
<td>79.1 ±2.4</td>
<td>36.9 ±1.6</td>
<td>66.9 ±2.4</td>
<td>0.98 ±0.12</td>
</tr>
<tr>
<td>5</td>
<td>SQ</td>
<td>25.7 ±1.3</td>
<td>&lt;1%</td>
<td>79.9 ±1.5</td>
<td>33.6 ±1.6</td>
<td>66.7 ±1.6</td>
<td>0.93 ±0.08</td>
</tr>
</tbody>
</table>

Values in the same category with common superscripts are not significantly different by Duncan's Multiple Range test. Values with no superscripts are not significantly different from either group.

Values in the table are means of four to six replicate observations.

No significant difference was shown to exist during the two periods and are not consistent. Whereas the difference in apparent absorption of magnesium for rats fed Diet T in Period 1 and for rats fed this diet in Period 2 is only 0.8%, the corresponding difference for rats fed Diets C, D, and H is 11.9, 9.1, and 12.7, respectively. It can be seen that these differences are likewise not well related to the levels of magnesium in the various milk diets, since all of them contained from 0.43-0.52% of magnesium, amounts regarded adequate for rats (11).

No significant difference was shown to exist between magnesium balance for rats fed dried whole milk and any of the phosphate-containing sterile milk concentrates during Period 1; for Period 2 a significant difference existed for only one of the three groups of rats fed phosphate-containing sterile concentrates. For both Periods 1 and 2 the mean apparent absorption of magnesium was highest for rats fed dried whole milk; however, these values were significant when compared to only one of the three groups fed diets containing phosphate-stabilized sterile concentrated milk.

It is possible to conclude that young growing rats are able to utilize the phosphorus, calcium, magnesium, and proteins of sterile concentrated milks containing polyphosphates as well as can rats fed spray-dried whole milk or concentrated milk without added polyphosphates. No evidence was obtained which indicates that polyphosphates in sterile milk concentrates have a deleterious influence on nutritive value of the milk as determined by balance and apparent absorption of phosphorus, calcium, and magnesium and by biological value and digestibility estimations for protein utilization.

Acknowledgments

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References

(6) Forbes, R. M. Personal communication.