FURTHER STUDIES ON THE GOITROGENIC EFFECTS OF PEARL MILLET DIETS

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ABSTRACT

For some parts of Africa there is a strong, positive correlation between goiter incidence and per capita millet production. Results of this study show that rats fed pearl millet diets tend to develop symptoms similar to those of colloid goiter in humans. Autoclaving the grain appears to alleviate the symptoms, but iodine supplementation does not. Changes in thyroid hormone levels in millet-fed animals cannot be attributed to high dietary iron content, but iron intake might be associated with certain histological changes in thyroid tissue.

INTRODUCTION

The World Health Organization (WHO) has reported that in some areas of Africa endemic goiter is a major public health problem (1). Insufficient dietary iodine is probably the most important cause of the problem, but the presence of goitrogenic substances in staple foods is also a significant factor (2). Surveys taken in the Darfur area of Sudan, where pearl millet (Pennisetum americanum (L.) Leeke) is a dietary staple, showed that goiter incidence was directly related to millet consumption when iodine intake was the same (3). When WHO estimates of goiter incidence in six African countries are compared with their per capita millet production, a direct relationship is observed (Fig. 1). The correlation coefficient (r=0.968, P=0.002) is amazingly high, considering the difficulty in obtaining accurate estimates of goiter incidence and millet production.

Previous work in our laboratory has shown that histological changes do occur in thyroid tissue of millet-fed rats, and we have noted thyroid hormone abnormalities in their blood serum (6). A goitrogenic agent which is not destroyed by fermenting the grain, is apparently associated with both bran and endosperm fractions (6). Very high negative correlations were noted between serum triiodothyronine (T3) concentrations and dietary iron, copper, and zinc (6). It was the goal of the present experiment to further define the characteristics of the pearl millet goitrogen by answering the following questions: Does iodine supplementation reduce the goitrogenicity of pearl millet diets? Is the goitrogen destroyed by heat? Is the high iron content of some pearl millet diets responsible for thyroid malfunction?

1Contribution No. 83-268-J, Department of Grain Science and Industry, Kansas Agricultural Experiment Station, Kansas State University, Manhattan, KS 66506, USA.
Figure 1. Relationship between per capita pearl millet production (pounds/person/year) and percent goiter incidence. Millet production figures were taken from Hulse et al (4), population data from English (5) and goiter incidence values from DeMaeyer et al (1).

MATERIALS AND METHODS

Five groups of 10 or 11 male, Wistar rats each were fed the following experimental diets for six weeks:

1. Pearl millet diet - Whole grain (13.8% protein), grown at the Agricultural Experiment Station in Hays, Kansas, was ground in a Ross Experimental Mill with corrugated rolls set at 0.016 inch.

2. Pearl millet + iodine diet - The same whole grain as for Diet 1 was used, similarly ground. Potassium iodide was added to the rats' drinking water to a level of 30 ppm of iodine.

3. Autoclaved pearl millet diet - The ground millet was heated in an autoclave at 15 psi for 20 minutes.

4. Sorghum grain (Sorghum bicolor, (L.) Moench) diet - Locally grown, low-tannin grain sorghum (8.9% protein) also was ground in the Ross Experimental Mill with corrugated rolls set at 0.016 inch.

5. Sorghum grain + iron diet - This was the same as Diet 4 except that 200 ppm of iron in the form of ferric citrate was added to the rats' drinking water.

All diets and drinking water were freely available to the animals throughout the experiment. Rat were housed individually and weighed 189 ± 10 grams after the three-day acclimatization period during which they were
fed Purina Rat Chow. Iodine content for both grains was nearly ten times higher than the National Research Council's minimum requirement of 0.15 ppm (7). Animals were weighed at weekly intervals, feed-consumption records were kept, and feed-efficiency ratios were calculated. After six weeks, blood samples were taken by heart puncture from ether-anesthetized rats. Serum was prepared by allowing the blood to clot at room temperature, then centrifuging in the cold (5°C) at 3000 x g for 30 minutes. Serum samples were kept frozen in dry ice until being radioimmunoassayed for thyroxine (T4) and triiodothyronine (T3). Tracheal segments with adhering thyroid glands were removed and preserved in 10% buffered, neutral formalin solution. Later, they were sectioned, stained, and microscopically evaluated. Analysis of variance procedure with Duncan's Multiple Range Test was used to statistically analyze the data (8).

RESULTS AND DISCUSSION

Weight gain. Addition of iodine to the diet of pearl millet-fed animals did not significantly affect their rate of weight gain (Fig. 2).

Figure 2. Weight gain curves: (a) pearl millet diet, (b) pearl millet + 30 ppm iodine, (c) autoclaved pearl millet, (d) sorghum grain, and (e) sorghum grain + 200 ppm iron.

Rats fed autoclaved millet grew slower than those fed the raw millet diets. After the fourth week, animals fed all three millet diets stopped gaining weight. With smaller rats we noted the same abrupt halt of weight gain after only two weeks of millet feeding (6). This is apparently the animals' response to mineral and/or vitamin deficiencies in the millet diets. Although their feed consumption fell steadily throughout the experiment, the animals appeared healthy and remained active.

Sorghum-fed rats grew slower than millet-fed animals during weeks 1-4 of the experiment, possibly because of the lower protein and energy contents.
of the sorghum grain. However, after week 4 these animals continued to gain weight. By the end of the sixth week, rats fed sorghum diet weighed the same as those fed the millet diets ($P < 0.05$). Table I shows that both grains contained far too little calcium for maximum weight gain, with pearl millet providing only 6.5% as much as needed and sorghum, 13.4%. The sodium content of both grains was also much below the NRC requirement (7).

Table I. Mineral content of grains used in experimental diets.

<table>
<thead>
<tr>
<th>Mineral*</th>
<th>Pearl Millet (ppm)</th>
<th>Grain Sorghum (ppm)</th>
<th>Rat requirement for maximum growth (7) (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium</td>
<td>162</td>
<td>336</td>
<td>2500</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>3489</td>
<td>2933</td>
<td>2000</td>
</tr>
<tr>
<td>Sodium</td>
<td>55.4</td>
<td>78.2</td>
<td>500</td>
</tr>
<tr>
<td>Potassium</td>
<td>2757</td>
<td>2231</td>
<td>1700</td>
</tr>
<tr>
<td>Copper</td>
<td>9.8</td>
<td>8.3</td>
<td>5</td>
</tr>
<tr>
<td>Zinc</td>
<td>29.4</td>
<td>29.9</td>
<td>12</td>
</tr>
<tr>
<td>Iron</td>
<td>43.1</td>
<td>44.5</td>
<td>35</td>
</tr>
</tbody>
</table>

*Assays performed using a Jarrell-Ash atomic absorption/emission spectrophotometer after dry-ashing of samples.

Supplementing the sorghum diet with iron significantly reduced weight gain rate (Fig. 2). Although those animals grew slower, they did continue to gain weight throughout the six-week period.

While feed efficiencies fell for all dietary groups during the experiment, the decline was much smaller for sorghum-fed groups than for those fed millet (Table II). Initially, the pearl millet was a much more efficient feed than sorghum, but after six weeks, sorghum was the more efficient feed.

Table II. Initial and final feed efficiencies (grams gained/gram of feed consumed)

<table>
<thead>
<tr>
<th>Diet</th>
<th>Week 1</th>
<th>Week 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pearl millet</td>
<td>0.191a</td>
<td>-0.041a</td>
</tr>
<tr>
<td>Pearl millet + iodine</td>
<td>0.193a</td>
<td>-0.043a</td>
</tr>
<tr>
<td>Autoclaved pearl millet</td>
<td>0.153b</td>
<td>-0.036b</td>
</tr>
<tr>
<td>Sorghum grain</td>
<td>0.095c</td>
<td>0.078b</td>
</tr>
<tr>
<td>Sorghum grain + iron</td>
<td>0.077c</td>
<td>0.065b</td>
</tr>
</tbody>
</table>

Means in the same column not followed by the same superscript are significantly different at $P < 0.05$.

Thyroid hormone concentrations. Rats fed the raw millet diets had
higher T4 levels and lower T3 levels than those fed grain sorghum, but animals fed the autoclaved millet had the same thyroid hormone pattern as the sorghum-fed animals (Table III). Intense heating of the millet

Table III. Thyroid hormone concentrations in rat serum.

<table>
<thead>
<tr>
<th>Diet</th>
<th>Thyroxine (T4) (ng/ml)</th>
<th>Triiodothyronine (T3) (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pearl millet</td>
<td>50.4b</td>
<td>1.11b</td>
</tr>
<tr>
<td>Pearl millet + iodine</td>
<td>58.7a</td>
<td>1.13b</td>
</tr>
<tr>
<td>Autoclaved pearl millet</td>
<td>45.7bc</td>
<td>1.33a</td>
</tr>
<tr>
<td>Sorghum grain</td>
<td>44.3bc</td>
<td>1.47a</td>
</tr>
<tr>
<td>Sorghum grain + iron</td>
<td>41.9c</td>
<td>1.42a</td>
</tr>
</tbody>
</table>

Means in the same column not followed by the same superscript are significantly different at p < .05.

appears to, at least partly, destroy the millet goitrogen, indicating that it is probably either organic and/or volatile in nature. In rapeseeds (Cruciferae family, Brassica genus) enzymic hydrolysis of thioglucosides releases goitrogenic compounds (9). Soybeans contain a heat labile, low molecular weight glycopeptide which has been associated with goiter incidence (10). Similar compounds could be present in pearl millet. Supplementing the millet diet with iodine resulted in increased T4 concentrations, but serum T3 levels were not significantly increased, lending support to our earlier suggestion (6) that the millet goitrogen might act by preventing the deiodination of T4 to T3.

Histological evaluation of thyroid tissue. As in our earlier experiment (6), millet feeding resulted in enlarged thyroid colloid follicles with flattened epithelial cells, especially in the periphery of the gland (Fig. 3a). Thyroid follicles of iodine-supplemented animals were similarly affected (Fig. 3b). Autoclaving the millet did not eliminate the problem, although follicle distortion appeared to be less severe in some rats fed the autoclaved grain than in those fed raw millet (Fig. 3c).

Sorghum-fed rats had normal appearing thyroid follicles which were much smaller and more uniform with cuboidal epithelial cells (Fig. 3d). Supplementation of iron to sorghum-fed rats also resulted in enlargement of colloid follicles, but to a much lesser degree than millet feeding (Fig 3e). Excess intake of minerals, including iron, has been associated with high incidence of goiter (11). Earlier, we noted a high negative correlation between serum T3 levels and dietary iron content (6). It appears from the present experiment that, while iron intake did not significantly affect thyroid hormone patterns, some histological changes did occur in iron-supplemented animals, and we cannot rule out high iron consumption as a possible factor in goiter etiology. Parathyroid glands of both sorghum- and millet-fed animals were greatly enlarged, reflecting the severe calcium deficiencies of the grain diets.

In rats, as in humans, T3 can arise from deiodination of T4 in tissues other than the thyroid (12). It is well known that peripheral conversion is depressed significantly in patients with liver disease (12) and other acute illnesses (13, 14), as well as in cases of protein-calorie malnutrition (15). By the end of the sixth week of the present experiment, it was obvious that all the millet-fed rats, including those fed autoclaved
Pearl millet, were undernourished. Their feed consumption had fallen much below that of sorghum-fed animals (88 g/rat/week vs. 145 g/rat/week), and some were losing weight, so the decreased T3 levels might have been attributed to their poor nutritional status, except that the hormone patterns of the rats fed autoclaved millet were the same as those of sorghum-fed animals. Histologically, the thyroid colloid follicles of rats fed autoclaved millet appeared to be less distorted than those of animals fed raw millet. This is positive evidence that the goitrogenic effect of pearl millet was not a result of malnutrition, but of a heat-labile component of the grain. These results also help to explain the strong, positive correlation between per capita consumption of millet and goiter incidence.
ACKNOWLEDGMENT

This research was supported by the INTSORMIL Title XII sorghum and millet research program (Grant AID/DSAN/XII-G-0149).

REFERENCES


Accepted for publication: August 24, 1983.