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THE GOITROGENIC EFFECT OF TWO SUDANESE PEARL MILLET CULTIVARS IN RATS #

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ABSTRACT

Pearl millet (Pennisetum americanum L. Lecke) is the staple food in many goiter areas in the west of Sudan while wheat is an additional staple food in low goiter areas. Epidemiological evidence from these areas suggests that although iodine deficiency is the major cause of goiter, millet consumption may play a role in goiter causation. This study was designed to determine in rats the possible goitrogenic and antithyroid effects of two millet cultivars, Bayoda and Balady, consumed in two endemic goiter areas with different goiter prevalence rates in the west of Sudan. Both fermented and unfermented forms of the two cultivars of millet were included in the study. Whole grain millet and wheat used in this experiment were only fortified with iodine with an amount supplying recommended dietary requirements. Feeding the two millet cultivars unfermented was associated with serum thyroid hormones comparable with the wheat reference while feeding the Balady cultivar (consumed in the area of low goiter prevalence) unfermented was associated with significant enlargement of the thyroid gland. Fermentation, was associated with the same trend of changes in both cultivars: enlargement of the thyroid gland and increased serum T4, T3 and TSH. Compared to the wheat reference group, fermentation of the cultivars was associated with increased serum T3 and T4 (only Bayoda) and significant further enlargement of the thyroid gland (only Balady). Among the animals that consumed millet (two way analysis), those receiving fermented millet showed increased serum T4, T3 and TSH compared to those which received unfermented millet. Nutritional inadequacies reflected in impaired growth, and enlarged heart and kidneys were more pronounced in animals fed millet diets than in those fed the wheat reference diet. In conclusion, in rats, the consumption of millet interferes with thyroid function; the consumption of Balady seems to induce an enlargement of the gland whereas the consumption of Bayoda causes modifications in the pattern of thyroid hormones.

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Key words: Fermentation, Goitrogen, Millet, Sudan, Thyroid, propylthiouracil (PTU)

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About 10 % of the world population have goiter. Many studies have shown that iodine deficiency is the major etiological factor in the causation of goiter (1). However high incidence of goiter also exists where iodine deficiency is not a problem (2,3). Moreover different prevalence rates of goiter have been found in areas lying in the same ecological zone (4). It is also noted that goiter occurs in 100 million people in highly developed countries, despite iodine prophylaxis (5). These and other observations suggest a potential role of dietary goitrogen in the causation of goiter (6,7). In fact some foods, like cabbage, rutabaga, turnips, and cassava have been designated as goitrogenic (6). The compounds which are responsible for the goitrogenicity of these plants, are primarily thioglucosides or cyanoglucosides (6). The contribution of thiocyanate overload to the high incidence of myxedematous cretinism in Ubangi, Zaire has been reported (6,7).

In western Sudan in areas with high prevalence of goiter (55–75 %), fermented millet is the main source of dietary energy while in areas with low prevalence of goiter (13 %), beside millet people consume other cereals like wheat and sorghum (4). It was concluded that millet might be goitrogenic and the contribution of other factors to the prevalence of goiter has been suggested (4,8). These include protein energy malnutrition, low vitamin A intake, and high sodium, potassium and iron content in drinking water. Moreover a cross sectional study made in a village in the same region in the west of Sudan showed increased frequency of juvenile hypothyroidism in children above two years of age compared to children below two years (9). This increased frequency has been attributed to millet consumption as a weaning food. It is reasonable to speculate that goitrogens in millet could be metabolized by the human body and therefore could not be detected in the milk of lactating mothers in large concentrations. However, the presence of certain phenolic compounds similar to those generated after fermentation of millet have been detected in breast milk specimens collected from western Sudan (Khalid SA, unpublished data). Thyroid histological changes similar to those observed in human colloid goiter were found in Purina rats fed millet diets (8). Noteworthy was the observation that rats consuming millet stopped growing after two weeks of feeding while they remained alert and active. When combining sorghum bran with millet endosperm, the animal growth was significantly greater than when feeding whole millet. Millet diets were shown to be rich in C-Glycosylflavones which are present in highest concentration in the bran and consequently reduced by higher extraction rate (10). The goitrogenic and antithyroid activity of these millet based diets was demonstrated in vivo, particularly glucosylvitexin, the major C-Glycosylflavone of millet, at a concentration of 60 μmol/L produced effects similar to those induced by 1 μmol/L methylmercaptoimidazole (MMI) in vitro (10). Based on these observations the consumption of millet associated with iodine deficiency was incriminated in goiter causation (10). Interestingly the two millet cultivars used in this experiment were different in color, one was slightly darker (Balady) than the other (Bayoda). The color of millet has been associated with the presence of certain phenolic compounds (11).

The aim of our work was to study in rats the relative effects of two different cultivars of millet usually consumed in two areas with different goiter prevalence rates in Sudan. To achieve these goals we compared the effects induced by these two cultivars of millet on goiter size and thyroid function to those produced by standard diets (casein or wheat). It is worthwhile mentioning that particular attention, during this work, was paid to the use of unfermented and fermented millet diets as they are prepared by people in Sudan. Liver and other organs were retrieved to investigate possible nutritional inadequacies and toxic effects not related to goitrogenicity.
MATERIALS AND METHODS

PREPARATION AND ANALYSIS OF DIETS.

The energy and protein contents of the diets used in the study is shown in Table 1. A casein diet regularly used as control in bioavailability studies, was compared to a wheat reference diet and to four experimental diets made from two cultivars of pearl millet called Bayoda and Balady, obtained from two rural areas in Sudan. These two cultivars were botanically identified as *Pennisetum americanum* L. Leek (I. Hedberg and O. Hedberg personal communications). The standard diet (G1, control group) was a synthetic diet made of casein mixed with oil, starch, minerals and vitamins (12) and its protein content was adjusted to be similar to that of experimental diets. This diet (G1) was used as an overall control for the whole biological assay. A dose of 5 ppm propylthiouracil (PTU) was added to casein diet (G2). For the reference group (G3), the diet was made of whole wheat flour. This wheat diet (G3) is used as a reference diet because it is a cereal and approximately similar to millet composition. Furthermore, wheat is the staple food in low goiter areas in western Sudan. The experimental diets (G4-G7) were prepared from whole millet flour ground twice in a roller mill; diets G4 and G6 with unfermented millet and diets G5 and G7 with fermented millet. Diets 3 to 7 were cooked with deionized water into porridges in a manner similar to what is traditionally done by local people. The porridges were lyophilized and ground in a turbmix. The moisture content of all diets was determined by drying at 105°C to constant weight. All diets were fortified with potassium iodide (0.16 mg iodine/Kg dry matter) irrespective of the endogenous amount of iodine present in the diets. The amount of iodine added was equal to the daily requirement for rats (13). The diets were kept in a freezer at -20°C. The protein content of the diets was analyzed by the Kjeldahl technique using Kjeltrec® Auto Analyzer (Model 1030 from Perstorp Analytical, Tecator AB, Sollentuna, Sweden) and converting nitrogen into crude protein by multiplying the results by 6.25. Analysis of energy in the diets was performed with a Parr® Adiabatic calorimeter (model 1241 from Parr Instrument Company, Illinois 61265, USA). The casein and millet diets were approximately isocaloric on the basis of dry matter. The wheat diet had 18% less caloric value compared to the casein diet.

![Table 1](attachment:table1.png)

Protein and Energy Content of the Diets Used in the Experiment. Values for Protein and Energy Contents are Given per 100 g Dry Matter of Diet.
FEEDING OF ANIMALS AND SAMPLE COLLECTION.

Forty six male weanling (25 days old) Sprague Dawley rats were housed in metabolic cages. After a three days acclimatization period during which the animals were given free access to the casein diet (G1) and deionized water, the animals were divided into 7 groups (5 to 8 animals in each group, see Table 1) in a way that there were no significant difference in the mean initial weights between groups. Mean weight of rats was 83.5±4.2 grams. Thereafter each group of rats was given the control or experimental diets as shown in Table 1. The food intake was restricted to an amount equivalent to 10 grams of dry matter per day per animal in order to standardize the experimental procedure. The animals were fed for a total period of 30 days with 12 hours dark and light cycles; the temperature was 21°C and the humidity was 50%. Free access to deionized water was given throughout. On the last day of the feeding period each animal was weighed in the morning and anesthetized by diethylether. Blood was collected by aortic puncture and serum was separated for further estimation of biochemical parameters of thyroid function (T3, T4 and TSH). The thyroids, liver, kidneys and heart were carefully dissected and immediately weighed. As the animals in the different groups did not show the same growth pattern the weight of these organs were expressed per 100 grams of final body weight.

ANALYSIS OF SERUM THYROID HORMONES AND THYROTROPIN.

Serum T4, T3 and TSH were determined according to methods previously described (14). For serum T4: 200 μl 125I-T4 and 300 μl rabbit antiserum to T4 both in barbital buffer were added to 20 μl of serum. After overnight incubation at 4°C bound and free fractions were separated using antirabbit gamma globulins. For serum T3: 200 μl 125I-T3 and 300 μl rabbit antiserum to T3 both in barbital buffer were added to 50 μl of serum. After overnight incubation at 4°C the free and bound fractions were separated using antirabbit gamma globulins. For serum TSH: 200 μl 125I-TSH and 100 μl of rabbit antiserum to r-TSH both in phosphate buffer were added to 100 μl of serum. After incubation for 24 hours at room temperature, bound and free fractions were separated using antirabbit gamma globulins. The reagents for this assay were provided by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) through the National Hormone and Pituitary Program (Dr. S. Raiti).

STATISTICAL ANALYSIS

The statistical analysis was performed using a computer program (Statpro® from Penton Software Inc. New York, N.Y. 10017 USA). Analysis of variance was performed by Duncan's multiple range test (15) improved by Harter (16). The relationship between different variables was explored by regression analysis. Two way unequal classification was also made to investigate possible differences and interactions between different parameters. In the tables the results were presented with the significance level P<0.01. Lower or higher significance level was presented only in the text.

RESULTS

WEIGHT GAIN

Table 2 summarizes in the different study groups: 1) mean protein consumed, 2) mean energy consumed, 3) mean total weight gain and 4) mean weight gain/1000 KJ consumed. The mean consumption of energy and protein were significantly higher in the two groups fed casein diets (G1
The mean weight gain at the end of the feeding period was significantly lower when feeding millet diets (G4 to G7) in comparison to feeding wheat (G3) which in turn was significantly lower in comparison to feeding casein (G1 & G2). Animals which consumed millet diets were 15-18% lighter than animals that consumed wheat, and 42-44% lighter than those consuming casein diets. The relation of weight gain in animals fed millet diets to those fed wheat diet was the same no matter whether weight gain was expressed in grams or in grams/1000 kJ consumed.

**TABLE 2**

Protein and Energy Consumed, Absolute Weight Gain and Weight Gain per 1000 kJ Consumed in the Experimental Groups (Mean±SD).

<table>
<thead>
<tr>
<th>Group No</th>
<th>Protein consumed (g)</th>
<th>Energy consumed (kJ)</th>
<th>Absolute weight gain (g)</th>
<th>Weight gain g/1000 kJ consumed</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>34.3±0.0 a</td>
<td>6456±0 a</td>
<td>96.5±7.4 a</td>
<td>15.0±1.2 a</td>
</tr>
<tr>
<td>G2</td>
<td>34.3±0.0 a</td>
<td>6456±3 a</td>
<td>96.5±4.2 a</td>
<td>15.0±0.6 a</td>
</tr>
<tr>
<td>G3</td>
<td>31.5±0.9 ab</td>
<td>4805±142 b</td>
<td>31.7±6.4 b</td>
<td>6.6±1.2 b</td>
</tr>
<tr>
<td>G4</td>
<td>28.8±1.8 bc</td>
<td>4739±295 b</td>
<td>20.8±6.0 c</td>
<td>4.3±1.0 c</td>
</tr>
<tr>
<td>G5</td>
<td>24.3±1.7 d</td>
<td>4666±329 b</td>
<td>17.1±8.2 c</td>
<td>3.7±1.9 c</td>
</tr>
<tr>
<td>G6</td>
<td>27.9±1.3 c</td>
<td>4854±224 b</td>
<td>20.1±6.1 c</td>
<td>4.1±1.1 c</td>
</tr>
<tr>
<td>G7</td>
<td>27.0±3.6 c</td>
<td>5105±688 b</td>
<td>17.8±7.1 c</td>
<td>3.6±1.5 c</td>
</tr>
</tbody>
</table>

Means that have the same letter are not significantly different (P>0.01). For information of diet in each group see Table 1.

**EFFECTS ON THE WEIGHT AND FUNCTION OF THE THYROID.**

Table 3 summarizes the mean thyroid weight in mg/100 gram body weight and mean serum T4, T3, and TSH in the study groups. Figure 1 shows these parameters in percent of the casein control group (G1).

**EFFECTS ON THE WEIGHT OF THE THYROID GLAND (Fig. 1a).**

Compared to G1, PTU given with the casein diet (G2) was associated with slight but not significant enlargement of the thyroid gland. Among all groups the mean thyroid weight of rats that received fermented Balady millet (G7) was significantly greater than any other group. In rats receiving fermented millet (G5 & G7), the mean thyroid weight was greater than in the corresponding groups fed unfermented millet (G4 & G6). The difference was highly significant only for the Balady cultivar (G7). For the later cultivar, in rats fed unfermented millet (G6) the mean thyroid weight was also significantly higher (P<0.05) than in the group of rats receiving wheat reference diet (G3) or unfermented Bayoda millet (G4). The weight of the thyroid gland did not correlate with final body weight.

**EFFECTS ON SERUM LEVELS OF T4 (Fig. 1b).**

In G2 the administration of PTU was associated with a marked and significant reduction in mean serum T4 (P<0.05). In animals which had enlarged thyroid i.e. that received fermented (G7) and
unfermented (G6) forms of Balady millet, serum T4 was not significantly different from the reference group (G3). By contrast, the mean serum T4 of animals that received the fermented form of Bayoda millet (G5) was significantly higher than the values observed for groups G1 to G4 and G6 (P<0.01) and for the group receiving fermented Balady millet (G7; P<0.05). In the group of animals that consumed the fermented form of Balady millet (G7), regression analysis showed a significant positive correlation between the thyroid weight and serum T4 (r=0.96, P<0.01); such significant correlation could not be demonstrated in any other group.

EFFECTS ON SERUM TSH (Fig. 1c).

Only animals that received PTU (G2) had mean serum TSH significantly greater than other groups. Animals that received the millet diets (G4 to G7) showed serum TSH comparable to that of the reference animals (G3). Other statistical methods (two way unequal classification) showed that rats fed fermented millet diets (G5 and G7), had significantly higher serum TSH compared to those fed unfermented millet (G4 and G6) (P<0.02).

EFFECTS ON SERUM T3 (Fig. 1d).

The administration of PTU was associated with low serum T3 (P<0.05). All other groups were comparable with the exception of G5 and G7 in which animals consuming fermented millet diets showed mean serum T3 significantly higher than all other groups.

**TABLE 3**
Thyroid Weight and Serum T4, T3 and TSH in the Experimental Groups (Mean±SD).

<table>
<thead>
<tr>
<th>Groups No</th>
<th>Thyroid weight mg/100 g BW</th>
<th>Serum T4 ng/ml</th>
<th>Serum T3 ng/ml</th>
<th>Serum TSH ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>4.0±0.8 b</td>
<td>52.7±6.0 bc</td>
<td>0.92±0.10 c</td>
<td>2.7±1.2 a</td>
</tr>
<tr>
<td>G2</td>
<td>4.9±1.1 b</td>
<td>32.0±7.9 c</td>
<td>0.65±0.09 c</td>
<td>13.4±6.3 b</td>
</tr>
<tr>
<td>G3</td>
<td>3.7±0.8 b</td>
<td>68.7±6.7 b</td>
<td>1.30±0.22 bc</td>
<td>2.5±1.2 a</td>
</tr>
<tr>
<td>G4</td>
<td>3.7±1.0 b</td>
<td>69.6±7.8 b</td>
<td>1.14±0.33 c</td>
<td>2.1±0.8 a</td>
</tr>
<tr>
<td>G5</td>
<td>4.4±1.8 b</td>
<td>95.0±27.5 a</td>
<td>2.07±0.54 a</td>
<td>2.6±1.3 a</td>
</tr>
<tr>
<td>G6</td>
<td>5.5±1.6 b</td>
<td>58.4±18.8 b</td>
<td>1.16±0.40 bc</td>
<td>1.3±1.1 a</td>
</tr>
<tr>
<td>G7</td>
<td>8.3±2.2 a</td>
<td>70.6±11.4 ab</td>
<td>1.99±1.00 b</td>
<td>3.5±1.6 a</td>
</tr>
</tbody>
</table>

Means that have the same letter are not significantly different (P>0.01). For information about diet in each group see Table 1.

EFFECTS ON OTHER ORGANS.

Table 4 shows the mean weights of the liver, heart and kidney per 100 gram of body weight. The mean liver weight/100 g BW in the millet groups were significantly lower than in the wheat reference group (G3). According to two way unequal classification, the groups receiving fermented millet (G5 & G7) had significantly lower liver weight compared to groups receiving unfermented millet (G4 and G6; P<0.001).
FIG 1. Thyroid, Weight and Serum T4, TSH and T3 in the Study Groups in Percent of the Casein Control Group (G1)
The mean heart weight was significantly greater in millet groups (G4 to G7) than in the casein groups (G1 and G2). Fermented Balady millet (G7) induced the highest mean heart weight. The mean weight of the kidney have approximately similar pattern as the weight of the heart.

**TABLE 4**
Liver, Heart and Kidney Weight in g/100 g Body Weight in the Experimental Groups of Rats (Mean±SD).

<table>
<thead>
<tr>
<th>Groups No</th>
<th>Liver weight g/100 g BW</th>
<th>Heart weight g/100 g BW</th>
<th>Kidney weight g/100 g BW</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>3.08±0.17 bc</td>
<td>0.38±0.02 b</td>
<td>0.77±0.07 bc</td>
</tr>
<tr>
<td>G2</td>
<td>3.17±0.12 b</td>
<td>0.39±0.05 b</td>
<td>0.75±0.04 c</td>
</tr>
<tr>
<td>G3</td>
<td>3.43±0.26 a</td>
<td>0.46±0.06 ab</td>
<td>0.87±0.05 ab</td>
</tr>
<tr>
<td>G4</td>
<td>3.07±0.14 bc</td>
<td>0.50±0.04 a</td>
<td>0.92±0.08 a</td>
</tr>
<tr>
<td>G5</td>
<td>2.89±0.13 cd</td>
<td>0.51±0.06 a</td>
<td>0.91±0.06 a</td>
</tr>
<tr>
<td>G6</td>
<td>3.05±0.12 bcd</td>
<td>0.51±0.05 a</td>
<td>0.97±0.07 a</td>
</tr>
<tr>
<td>G7</td>
<td>2.79±0.10 d</td>
<td>0.52±0.06 a</td>
<td>0.92±0.06 a</td>
</tr>
</tbody>
</table>

Means that have the same letter are not significantly different (P>0.01).
For information of diet in each group see Table 1.

**DISCUSSION**

**EFFECTS ON WEIGHT GAIN**

With the exception of the casein groups (G1 and G2) the mean energy consumed (kJ) was very similar in the wheat reference group (G3) and the millet groups (G4 to G7). As the protein content of casein diet was adjusted to be like that of millet and wheat, the differences in weight gain/1000 kJ of energy consumed from the different diets were not due to quantitative differences in protein content. The weight gained /1000 kJ of energy consumed was not correlated to any of the effects observed on organs or hormones. In the wheat (G3) and the millet groups (G4 to G7) some other causes account for the reduction in weight gain/1000 kJ energy consumed. These include low protein quality, other nutritional inadequacies in wheat and millet and possible toxins in millet (8). Among the cereals used in Nigeria it has been shown that black and brown millet contained the highest oxalate and tannin levels, 4.65 mg/g, and 0.07 mg/g, respectively (17). These components may have antinutrient effects and make the plant proteins and trace minerals only partially available. Bulrush millet from Tanzania was shown to have low protein quality; biological value (BV) varied between 48-62% (18). Weight gain was strongly and positively correlated to BV. Lysine was the first limiting amino acid. It is well documented that BV is also quite low in wheat due to low lysine content and that this is the main cause of poor growth (19). The presence of toxins (aflatoxin) in food reduces the feed efficiency, and consequently the weight gain (20). Even if the presence of an unknown toxin cannot be ruled out it is assumed that low BV value could be the major determinant of growth impairment in the present two millet cultivars.
EFFECTS ON SIZE AND FUNCTION OF THYROID GLAND

Effects of PTU.

In this experiment, PTU given with casein did only induce slight but not significant increase in the mean thyroid weight. Interestingly in one study (21) given with a high protein diet (60% casein) another antithyroid agent, methyl-mercapto-imidazole (MMI), failed to induce significant enlargement of the thyroid gland while a slight but significant enlargement of the thyroid gland was induced when giving a low protein diet (8% casein). In the present study, our diet has 10.6% casein. Despite the absence of spectacular enlargement of the thyroid, the administration of PTU to rats fed casein resulted in a marked reduction of serum T4 and T3 and a striking rise in serum TSH. It should emphasized that these animals were not iodine deficient.

Effects of feeding unfermented millet.

With respect to feeding of unfermented millet to rats, only one of the two millet cultivars used in the experiment, namely the slightly dark Balady cultivar, was associated with significant enlargement of the thyroid gland (p<0.05). By mixing his millet diets with 30% of his control diet (Remington diet) which provided nutritional fortification, Gaitan (10) demonstrated a significant enlargement of the thyroid in female rats. This was only obtained with millet bran fractions rich in glycosylflavonoids but not with whole millet. By contrast in the present study, we could show significant enlargement when feeding whole millet. It is clear from the present study that different cultivars of millet have different effects and the use of a different strain of rats compared to Gaitan (10) might account for the difference. Gaitan also showed that heating of millet akin to the manner local people used to do, resulted in an increased goitrogenicity (11). This can be explained in terms of the generation or disappearance of certain metabolites (e.g certain phenolic compounds) during the process of cooking. In the present experiment our millet diets were cooked and this might have increased the goitrogenicity of our whole millet. Feeding the unfermented forms of both millet cultivars (G4 & G6) was associated with serum T4 and TSH comparable to those observed in the reference group (G3).

Effects of feeding fermented millet.

Fermentation of the goitrogenic Balady cultivar of millet potentiated its goitrogenic effect. Our observation indicates that fermentation is associated with an increase in the goitrogenic effect of millet as fermentation resulted in an increase of thyroid weight. This might result from the hydrolysis of the C-glycosylflavanoids during the fermentation process coupled with the breakdown of the glycones (22). Although fermentation of the Bayoda millet did not affect the size of the thyroid significantly it distorted the thyroid hormone profile by inducing a highly significant increase in serum T4 and T3 with TSH comparable to the control. Moreover fermentation of the goitrogenic Balady millet cultivar (G7) induced significant increase in serum T3, with serum T4 and TSH comparable to the control. A strong and positive correlation between serum T4 and the thyroid weight in animals receiving the fermented Balady millet (G7) was observed. This suggests that despite the increase in thyroid weight serum T4 and possibly T4 production were not modified. An action on the metabolic pathway involving the peripheral conversion of T4 to T3 might be an additional mechanism.
COMMENTS ON THE EFFECTS OF MILLET INGESTION ON THYROID HORMONES AND THYROTROPIN AND THE POSSIBLE MECHANISM OF GOITROGENESIS.

Increased serum levels of T4 and T3 have been observed in rats fed millet based diets (23). Fermented as well as unfermented millet diets also resulted in increased serum T4 but serum T3 was decreased insignificantly by the fermented form and significantly by the unfermented form (8). In the present study increase in both serum T4 and T3 were observed with fermented Bayoda millet (G5) and an increase in serum T3 alone with fermented Balady millet (G7). The unfermented forms of the two cultivars showed a slight but non significant decrease in serum T3. In previous studies (8, 23) as well as in the present study, whole millet was used unfortified except with iodine. The results of our study together with the previous ones (8, 23) suggest that the goitrogen in doses normally present in whole millet has little if any effect on thyroid hormone synthesis but possibly acts on the extrathyroidal metabolism of T4 and T3. In higher doses as the one present in bran fractions, the goitrogen seems to have a pronounced inhibitory effect on thyroid hormone synthesis (10). Ingbar reported a decreased T4 clearance with feeding goitrogenic millet bran fractions (unpublished observations cited in 10); this represents an evidence of possible extrathyroidal effects of millet. In the present study and the previous ones (8, 23) the reduction in T4 clearance might have obscured some of the direct inhibitory effects of the millet if any on thyroid hormones synthesis. However in the present study we cannot completely rule out an action on thyroid hormone synthesis. Indeed feeding the fermented Bayoda cultivar (G5) could result in a reduced T4 clearance causing a significant increase in serum T4 which could not be reproduced when feeding the fermented Balady millet (G7). In rats, low doses of the known antithyroid drugs like PTU and MMI are known to increase serum TSH with no or little effect on serum T4 concentration. Inhibition of the peripheral deiodination of T4 and/or reduction of the metabolic effectiveness of thyroid hormones can account for this observation (24). A decline in T4 is observed with higher doses of PTU and MMI (24). Another study in steers (25) showed that small doses of PTU caused an increase in serum T4 without concomitant modification in serum TSH. The high serum T4 observed in rats receiving fermented Bayoda millet (G5) might also result from the presence of a goitrogen in concentration sufficient to induce an increase in serum T4 but not to produce goiter. It has been shown that goiter can occur with different patterns of thyroid hormone profile; for instance, tetrachloro-tetraiodofluorescine sodium induces goiter with raised serum T4 and both normal T3 and TSH concentration (26). Various fractions of T4 and T3 have been advocated in the regulation of pituitary-thyroid axis e.g. total thyroxine, free thyroxine (27), peripheral deiodination rate of thyroid hormones (28), cellular exchange of T4 (29) or T3 (30). In addition, it has been shown that TSH secretion is not only regulated by the absolute level of thyroid hormones (28, 31), but also by the metabolic effectiveness and the rate of deiodination of thyroxine. Consequently goiter is expected to occur even with normal concentration of serum thyroid hormones if the goitrogen reduces the amount of thyroid hormone deiodinated at the cellular level.

The fact that the goitrous animals in this study have serum TSH comparable to controls was not surprising since Männistö (24) showed that in rats chronically treated with PTU or MMI, TSH rose to a peak by day 4 and declined to normal levels by day 14 while goiter occurred as early as day 4. Similarly, normal TSH with persistently low serum T4 were observed in rats fed millet bran fractions (10). So the presence of a goiter in rats consuming the Balady (G6 and G7) was possibly induced by a rise in TSH occurring early in the feeding period.

In conclusion, the two cultivars of millet used in the present study were shown to have different effects on thyroid function. Fermentation of Bayoda millet cultivar resulted in increased serum T4 and T3 while fermentation of the Balady millet cultivar resulted in high serum T3 and normal T4. Thin layer chromatographical investigation of fermented versus unfermented millet cultivars
revealed different chromatographical patterns therefore it is reasonable to associate these biochemical changes in serum T3 and T4 with chemical modification involving phenolic compounds in these cultivars (S. A. Khalid unpublished work). It seems that the goitrogenic substances were present in higher amounts in the Balady cultivar of millet. At such concentrations it probably has some inhibitory effects on thyroid hormone synthesis in rats, since T4 was not as high with the fermented Balady (G7) as with the fermented Bayoda millet (G5). The present study has shown that the dark Balady cultivar had clear-cut goitrogenic effects. Both cultivars exhibited their effects in the presence of iodine supplementation to dietary requirement. The possible goitrogenic effects of the two millet cultivars in the presence of iodine deficiency observed in the two consumption areas (32) is to be further evaluated. However it could be assumed that the two cultivars may contribute to the goiter prevalence rates in these areas.

Effects on other organs.

Compared to casein control and wheat reference, feeding millet to rats was associated with a trend suggesting a decrease in liver weight and an increase in the heart and kidney weight per 100 gram body weight. In previous experiments (unpublished data), wheat and millet induced organ effects were completely eliminated after dietary supplementation with proteins, oils, vitamins and minerals to requirement levels. The observed effects on heart weight and kidneys in this study were therefore also most probably associated with nutritional inadequacies. The different effects of wheat and millet on the liver weight could be related to different nutritional inadequacies. Indeed it is known that nutrient restriction reduces liver weight (33). The liver is the first organ to be exposed to dietary factors through the portal circulation and overall it is the most metabolically active organ. Most toxins cause enlargement of the liver if it is playing a role in their detoxification. Fermentation was associated with further reduction in the liver weight, despite the fact that fermentation increases the bioavailability of some nutrients (34). However we can further postulate that feeding fermented millet resulted in the exposure of the liver to less complex chemicals and therefore reduced the metabolic function of the liver and accordingly its weight in relation to feeding unfermented.

In conclusion, in rats, the effects induced by consumption of millet are multiple. These effects are modulated by several factors but namely by the cultivar and by the use of a fermentation process. The two cultivars used in the present study interfere with thyroid function in rats. Consumption of Balady variety mainly results in an increase of thyroid weight whereas that of Bayoda induces changes in the relative proportions of thyroid hormones.

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