

94% grain diets: 1) control (whole red sorghum grain); 2) whole gray pearl millet; 3) bran fraction of gray-seeded pearl millet; 4) red dog fraction of gray millet; 5) a mixture of flour, shorts, and red dog (37:47:16); 6); gray millet flour; 7) whole brown pearl millet; and 8) whole yellow pearl millet. Proximate compositions of the diets are shown in Table II. Each diet contained 2% of vitamin mixture No. 2 and 4% of USP XVII mineral mix, both of which are commonly used in biological test diets (ICN Nutritional Biochemicals, Cleveland, OH). The mineral mix contributed 1.76 mg of iodine per 100 g of diet. The contribution of iodine from the grains was small in comparison, because sorghum grain contains 0.004 mg/100 g (NRC 1982), and pearl millet contains 0.034 mg/100 g (Gaitan et al 1989). Millet bran (the outer 25% of the grain) is the fraction richest in iodine, containing about 0.091 mg/100 g (Gaitan et al 1989). Therefore, the iodine contents of the diets used in this experiment ranged from 1.76 to 1.85 mg/100 g, which was more than enough to meet the rats' requirements, but not so high as to cause changes in thyroid histology (Galton and Pitt-Rivers 1959).

After eight weeks, blood samples were taken by cardiac puncture from lightly ether-anesthetized rats. Serum was prepared and radioimmunoassayed for total thyroxine (T_4), triiodothyronine (T_3), free thyroxine (FT_4), and free triiodothyronine (FT_3). The rats were sacrificed by exposure to an ether atmosphere, and tracheolaryngeal sections with adhering thyroid glands were removed and placed in 10% buffered neutral formalin. Sections were embedded in paraffin, sectioned at 6 μ m, stained with hematoxylin and eosin, and evaluated microscopically.

RESULTS AND DISCUSSION

Weight Gain

As expected, weight gains of rats fed the different diets were quite different, depending on the protein and energy contents of the various grains and fractions (Table II). Weight gains of animals fed the flour-shorts-red dog diets were the same as those for animals fed the sorghum control diet.

Serum Total and Free T_4 and T_3 Concentrations

Total T_4 concentrations were generally lower in rats fed the

whole gray, brown, and yellow millets and the gray millet fractions than in those fed the sorghum control diet, except for animals fed the pearl millet flour diet (Table III). In those rats, T_4 values were not different from values for animals fed the control diet and were higher than those in animals fed any other millet diet. Because neither starvation nor overfeeding significantly affects serum T_4 or FT_4 levels (Cavalieri 1987), the differences in those values among groups can be attributed to thyrotoxic substances in the millets.

The effect of millet feeding was considerably greater on serum concentrations of total T_3 than on concentrations of total T_4 , although the trends were the same. Values were much lower in rats fed the whole millets and the gray millet bran and red dog fractions than in those fed sorghum. Both T_4 and T_3 concentrations were similar for rats fed the pearl millet flour or the flour-shorts-red dog diet and rats fed the control diet. Most (80%) of the circulating T_3 is not directly secreted by the thyroid gland but formed in peripheral tissues from deiodination of T_4 (Green 1987). The most common cause of reduced T_3 values is reduced conversion of T_4 brought on by a state of starvation or illness (Green 1987). However, that cannot be the reason for the low T_3 values found here, because the rats with the lowest concentrations were also the ones that were better nourished and gained more weight than those with higher T_3 levels. In studies with thyroidectomized rats supplemented with T_4 , Gaitan et al (1989) showed lower clearance of T_4 in rats fed millet fractions from the outer layers of the grain than in those fed interior fractions of the grain. Those workers also noted that intact rats fed millet fractions constituting 13.6 and 24.9% of the outer layers of the grain had decreased T_4 levels, which appeared to be associated with higher concentrations of flavonoid compounds in those fractions.

In contrast to the lower T_4 and T_3 levels found in millet-fed rats, FT_4 and FT_3 values and the proportion of free to total hormone were much higher than in the control animals, except

TABLE II
Proximate Compositions (db) of Experimental Diets and Overall Weight Gain in Rats^a

Diet	Crude Protein (%)	Crude Fat (%)	Ash (%)	Crude Fiber (%)	Overall Weight Gain (g)
Sorghum (control)	9.7	2.05	5.07	2.40	50.5 c ^b
Gray millet					
Whole	15.4	5.38	5.07	2.25	196.6 b
Bran	22.8	10.70	7.28	5.84	289.2 a
Red dog	13.0	6.33	5.21	4.33	195.6 b
Flour-shorts-red dog (37:47:16)					
Flour	12.0	3.60	4.40	1.84	66.0 c
Flour	8.5	1.66	4.24	0.95	21.8 d
Brown millet, whole	16.3	5.25	5.04	2.01	204.3 b
Yellow millet, whole	14.8	5.91	5.05	2.56	197.1 b

^a Twelve rats were fed each diet.

^b Means not followed by the same letter are significantly different at $P < 0.05$.

TABLE III
Total and Free Thyroxine and Triiodothyronine Levels^a in Serum of Rats Fed Various Diets and C-Glucosylflavone Concentrations of the Diets

Diet	T_4 (nmol/L)	FT_4 (pmol/L)	T_3 (nmol/L)	FT_3 (pmol/L)	FT_4/T_4 ($\times 10^{-4}$)	FT_3/T_3 ($\times 10^{-4}$)	Flavones (mg/100 g)
Sorghum (control)	117.6 ab ^b	12.4 e	3.18 a	2.57 d	1.09 de	8.1 d	0
Gray millet							
Whole	94.3 cd	37.7 c	2.08 c	4.10 bc	4.03 b	19.8 a	138.6
Bran	107.5 bc	47.8 a	1.68 d	3.80 bc	4.47 b	22.3 a	243.6
Red dog	84.5 d	34.4 c	1.70 d	3.80 bc	4.10 b	22.4 a	201.8
Flour-shorts-red dog (37:47:16)							
Flour	106.4 bc	14.7 e	3.20 a	2.94 d	1.44 d	9.4 d	97.0
Flour	124.3 a	11.4 e	3.43 a	1.95 e	0.92 e	5.7 e	48.6
Brown millet, whole	86.4 d	35.1 c	2.27 bc	4.74 a	4.17 b	20.8 ab	118.9
Yellow millet, whole	89.4 d	30.6 d	2.53 b	4.38 ab	3.51 c	17.0 c	147.2

^a T_4 = total serum thyroxine, FT_4 = free serum thyroxine, T_3 = total serum triiodothyronine, FT_3 = free serum triiodothyronine.

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

TABLE IV
Correlation of Serum Thyroid Hormone Concentrations^a
with C-Glucosylflavone Contents of Various Pearl Millet Diets

	T ₄	FT ₄	T ₃	FT ₃	FT ₄ /T ₄	FT ₃ /T ₃
<i>r</i> -value 1 ^b	-0.742 (0.091) ^c	0.913 (0.010)	-0.908 (0.012)	0.801 (0.055)	0.912 (0.010)	0.911 (0.012)
<i>r</i> -value 2 ^d	-0.650 (0.081)	0.887 (0.003)	-0.890 (0.003)	0.627 (0.096)	0.855 (0.007)	0.861 (0.006)

^aT₄ = total serum thyroxine, FT₄ = free serum thyroxine, T₃ = total serum triiodothyronine, FT₃ = free serum triiodothyronine.

^bCalculations include only gray millet diets.

^cNumbers in parentheses show probability.

^dWhole brown and yellow millets included in calculations.

for those fed the millet flour and flour-shorts-red dog diets, which had FT₄, FT₃, FT₄/T₄, and FT₃/T₃ levels similar to those of the control animals (Table III). That could be the result of either decreased amounts of available binding (transporting) protein or inhibition of protein binding. A number of drugs have been shown to competitively bind to T₄ or T₃ sites on thyroid hormone-binding proteins (Green 1987); compounds present in pearl millet may behave similarly.

When Reichert et al (1980) measured total C-glucosylflavone concentrations in 16 variously colored pearl millets, they reported values ranging from 87 to 259 mg/100 g. Values for the millets used in this experiment were all within that range (Table III).

All serum concentrations of thyroid hormones measured were highly correlated with C-glucosylflavone concentrations of the gray-seeded pearl millet diets (*r*-value 1, Table IV). FT₄, T₃, and FT₃ all appear to be better indicators of antithyroid activity in pearl millet than T₄, because correlations were higher for those values. In addition, FT₄ is the closest approximation of the level of thyroxine actually affecting the cells (Krupp et al 1979). This could be an important research consideration, because the radioimmunoassays are expensive and time-consuming. If resources permit only one assay, FT₄ seems the most appropriate.

When flavone values for the brown- and yellow-seeded pearl millets were included in the calculations, correlations were weaker in every case. This suggests that total flavone concentration is not the only variable affecting hormone levels and that composition of the flavone mixture is probably important also. As mentioned earlier, different degrees of antithyroid activity have been reported for glucosylvitexin, glucosylorientin, and vitexin (Gaitan et al 1989).

In general, the data show that gray, brown, and yellow millets all significantly affect serum concentrations of hormones. Total flavone concentration was higher in the yellow millet than in the other two millets. However, when significant differences in hormone concentrations or ratios occurred between rats fed the brown or yellow millets (FT₄, FT₄/T₄, FT₃/T₃, Table III), rats fed the yellow millet had levels closer to those of control animals, and rats fed brown millet had levels closer to those of animals fed gray millet.

Thyroid Histopathology

The thyroids of sorghum-fed rats and those of animals fed the gray-seeded pearl millet flour appeared normal. Colloid follicles were nicely filled with colloid; epithelial cells were cuboidal, and variation in follicle size was normal, with somewhat larger follicles on the perimeter of the glands. No evidence of hyperplasia was noted. Very minor abnormalities (lack of colloid and some evidence of hyperplasia) occurred in some animals fed the flour-shorts-red dog diet. In rats fed whole-ground gray, yellow, or brown millets and gray millet bran, follicles lacked colloid, and hyperplasia was common, with the most severe effects noted with the millet bran. The red dog diet also adversely affected thyroid histopathology, but not to as great an extent as did the whole millet or millet bran diets. With respect to degree of adverse

effects on thyroid histopathology, the diets are ranked in the following decreasing order: millet bran, whole millets, red dog, flour-shorts-red dog, and flour, which was the same as sorghum (no effect).

CONCLUSIONS

Gray, brown, and yellow pearl millets all had antithyroid activity, as shown by serum thyroid hormone levels and thyroid histological examination, although some effects appeared to be less severe in rats fed the yellow millet diet. Semiwet milling of gray-seeded pearl millet grain (in which 25% of the grain was removed as bran) successfully eliminated its antithyroid properties, as demonstrated by serum thyroid hormone patterns and thyroid histopathology. Removal of the protein and energy-rich bran also decreased the nutritional value of the pearl millet, although weight gains of the rats fed the flour-shorts-red dog diet were not significantly different from those of the control animals fed the sorghum diet. We found no changes in hormone patterns and very little difference in thyroid histopathology in pearl millet diets containing 97 mg/100 g or less of C-glucosylflavone. Therefore, pearl millet goitrogenicity can probably be reduced through appropriate selection and breeding programs. Until that is accomplished, the semiwet milling process offers an alternative for producing nongoitrogenic food from this dietary staple.

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