Semiwet Milling of Pearl Millet for Reduced Goitrogenicity[1]

C. F. KLOPFENSTEIN,[2] H. W. LEIPOLD,[3] and J. E. CECIL[4]

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ABSTRACT

It is well established that pearl millet grain (*Pennisetum glaucum:* syn., *P. typhoideum*, *P. americanum*) is goitrogenic, mainly because of its *C*-glycosylflavone content. Concentrations of the antithyroid compounds have been found to be much higher in the bran than in the endosperm portions of the grain. Traditional dry-roller milling processes are not very efficient at fractionating the grain because of its small size and firmly embedded germ. Therefore, semiwet roller milling was used to separate the grain into flour, shorts, red dog, and bran fractions. Rat-feeding studies showed that the semiwet milling process (in which 25% of the grain was removed as bran) successfully removed the antithyroid properties, as demonstrated by patterns of serum thyroid hormones and thyroid histopathology. Nearly all previous studies have used gray-seeded pearl millet. This study showed that brown- and yellow-seeded pearl millet also had antithyroid effects. Although flavone concentration was higher in the yellow than in the brown millet, antithyroid effects seemed somewhat less severe for the yellow millet, indicating differences in antithyroid potency and/or concentrations for the three *C*-glycosylflavones that have been identified in pearl millet grain.

Strong evidence suggests that the *C*-glycosylflavones and their metabolites in gray-seeded pearl millet (*Pennisetum glaucum:* syn., *P. typhoideum*, *P. americanum*) are goitrogenic (Birzer et al 1987, Birzer and Klopfenstein 1988, Gaitan et al 1989). Those compounds have also been identified as the agents that cause off-odors in ground pearl millet (Reddy et al 1986). The presence of another goitrogen, thiocyanate, has also been reported in pearl millet. However, its mechanism of action is different from that exhibited by pearl millet used in diets and extracts, and it is present in low concentration, so it has been discounted as the primary goitrogen in the grain (Gaitan et al 1989). Considerable epidemiological data indicate that the grain may be a significant cause of goiter in populations in which pearl millet is a dietary staple (Osman and Fatah 1981, Klopfenstein et al 1983).

In 1980, Reichert et al reported a steep gradient in *C*-glycosylflavone concentration from outer to inner portions of the seeds, with much higher percentages in the outer layers. However, because of the small kernel size and firmly embedded germ, efficient separation of the pearl millet bran and germ from the endosperm is difficult with a dry milling process. Semiwet roller milling processes have been used successfully to prepare low-fat grits from pearl millet (Abdelrahman et al 1983) and white flour from sorghum grain (Cecil 1987), but the effect of that type of milling on the goitrogenicity of pearl millet has not been established. In fact, the high-moisture milling process may have the potential for increasing the grain's goitrogenic properties, because evidence indicates that enzymatic processes activated when the grain is wet probably convert the flavonoid compounds in the grain to the known goitrogens, phloroglucinol and *p*-hydroxyphenylpropionic acid (Birzer et al 1987). Gaitan et al (1989) found increased antithyroid activity in aqueous buffer extracts of pearl millet that were heated and then stored.

In addition, most goitrogenicity studies have used gray-seeded pearl millet. Differences in pigmentation in pearl millet are probably the result of different concentrations of the three major *C*-glycosylflavones that have been identified in the grain (Reichert et al 1980). The three compounds have been found to vary in their ability to inhibit thyroid peroxidase activity. In decreasing order, they are glucosylvitexin, glucosylorientin, and vitexin (Gaitan et al 1989). The present study was designed to 1) determine the effects of semiwet roller milling on the goitrogenicity of gray-seeded pearl millet and 2) compare the antithyroid properties of gray-seeded pearl millet with those of yellow and brown pearl millets.

MATERIALS AND METHODS

Gray-, yellow-, and brown-seeded pearl millets were grown during the 1988 growing season, under nonirrigated conditions, at the Fort Hays Branch Experiment Station, Hays, KS. Sorghum grain (*Sorghum bicolor* (L.) Moench) grown during the same growing season was obtained from a local elevator in Manhattan, KS.

Semiwet roller milling was used with gray-seeded millet. The millet was tempered overnight to 26% moisture, then milled using a Buhler 202 laboratory mill with break and reduction roll settings and screens (Table I). Milling fractions obtained were: flour, 28%; bran, 25%; shorts, 35%; and red dog, 12%. Whole grains were not tempered to a higher moisture content before their particle size was reduced in a Ross experimental mill, with first break rolls set at 0.4 mm.

After a 10-day acclimatization period, 96 male weanling Wistar rats (12 per group), individually housed, were fed the following

[1]Contribution 90-580-J of the Kansas Agricultural Experiment Station, Manhattan, KS 66506.

[2]Department of Grain Science and Industry, Kansas State University, Manhattan, KS 66506.
[3]Department of Pathology, Kansas State University, Manhattan, KS 66506.
[4]9 School Place, Oxford, OX1 4RG England.

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 μm, 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 I
Configuration for Semiwet Milling of Pearl Millet Grain

Rolls		Screens			
Type	Gap Settings (mm)	Location	Size (μm)		
Break (B)		Break side			
B2	0.2	Top	437	437	297
B3	0.13	Bottom	130	130	130
Reduction (R)		Reduction side			
R1	0.07	Top	368	259	...
R3	0.03	Center	130	130	130
		Bottom	130	130	130

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)	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)	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
r-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)
r-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)

[a] T_4 = total serum thyroxine, FT_4 = free serum thyroxine, T_3 = total serum triiodothyronine, FT_3 = free serum triiodothyronine.
[b] Calculations include only gray millet diets.
[c] Numbers in parentheses show probability.
[d] Whole brown and yellow millets included in calculations.

for those fed the millet flour and flour-shorts-red dog diets, which had FT_4, FT_3, FT_4/T_4, and FT_3/T_3 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 T_4 or T_3 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). FT_4, T_3, and FT_3 all appear to be better indicators of antithyroid activity in pearl millet than T_4, because correlations were higher for those values. In addition, FT_4 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, FT_4 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 (FT_4, FT_4/T_4, FT_3/T_3, 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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