Effect of Combined Millet and Cabbage Balanced Diet on Thyroid Function in Albino Rats

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ABSTRACT

Goitrogens impair thyroid hormone synthesis but is not known if the goitrogen in millet will have antagonistic or synergistic effect on the goitrogen in cabbage. Hence the interactions between millet and cabbage was evaluated using different millet cabbage diet combination in rats. Millet (30%, 60% and 100%), Cabbage juice (30% and 60%), Standard diet were combined to give (10) feedings in three replicates with (2) rats per replicate, using a Randomized Block Design. At the end of 90 day, Serum TSH (mIU/ml) level was found to be significantly elevated ($p<0.001$) while Serum T_4 (ng/ml) levels was significantly reduced ($p<0.001$). Serum TSH and T_4 showed similar pattern for 30% cabbage juice and 30% millet diet on day 90 and day 45. Serum thiocyanate (ng/ml) levels in the treatment groups were not significantly altered ($p>0.01$). Histopathological analysis of the thyroid gland revealed hyperplasia in rats with 100% millet, while those on 60% and 30% millet were less affected. The results showed no induction of goiter by millet and cabbage but clear evidence of hypothyroidism as shown by elevated serum TSH and decreased serum T_4 levels.

KEYWORDS: Millet, Cabbage, Thyroid Function, Albino Rat.

INTRODUCTION

Goitrogens cause goitre and accordingly acquire their name from the word 'goitre,' which means the enlargement of the thyroid gland. These Goitrogens also affect the ability of the thyroid gland to synthesize its hormone leading to other thyroid diseases such as hypothyroidism and cretinism in both humans and animals (5,6). The isolation and identification of 1-5-vinyl-2-thiooxazolidine, a goitrogen of some foods in the Cruciferae family (1,2), led to the search for similar agents in more commonly consumed foodstuffs.

Another staple food implicated in the aetiology of goitre is pearl millet (Pennisetum americanum), after it has been observed that in rural Darfur province of Sudan, where pearl millet was a major staple, the incidence of goitre was higher than in urban regions where other food grains such as sorghum were consumed (9,10). Consumption of pearl millet is considered one of the factors responsible for the high incidence of goitre in rural populations. A positive correlation observed between the incidence of goitre and per capital production of pearl millet in six African countries appears to support this view (7,8). It has since been established that the goitrogenic principle of pearl millet could be extracted with alcohol and present as the c-glucosyl flavones (vitexin) and glucosyl orientin (3). Feeding trials in rats showed that the goitrogen inhibited deiodination of thyroxine (T_4) to triiodothyronine (T_3), the

metabolically more active form of the hormone. Iodine supplementation did not alleviate the goitrogenic effect of pearl millet (3,4).

In Northern Nigeria, there is high consumption of millet in different dietary forms and also consumption of cabbage grown in irrigated areas as locally produced salad. It is not known whether the goitrogen in millet will have antagonistic, additive or synergistic effect on the goitrogen in raw cabbage. Therefore this report investigates the possible combined effect of millet and cabbage goitrogens in rats.

2. MATERIALS AND METHODS

2.1 Preparation of Experimental Diets

Millet, Cabbage juice, Standard Chicken feed (chick mash; ECWA Rural Development LTD, Bukuru Jos, Nigeria) is the diets used in the study. These diets were varied as follows: Millet diet (30% millet mixed with 70% chick mash, 60% millet mixed with 40% chick mash and 100% pure millet). Cabbage juice was extracted from raw cabbage using an electric juice extractor and mixed as follows: (30% cabbage juice in 70% water and 60% cabbage juice in 40% water); these concentrations were then given to the rats daily in water bottles. Standard Chicken feed (chick mash or 0% millet). The diets were combined to give ten (10) treatments and these treatments are in three replicates, with two (2) rats per replicate separately housed in locally designed cages.

Sixty albino rats of mixed sex were purchased from the National Veterinary Research Institute, Vom. They were 20-30 days old and weighed between 70-100 g. the animals were ranked according to weight and paired (two rats per cage) according to sex and weight. They were then fed with experimental diets for ninety days. Each rat was first incubated at $45^{O}C$ for 5 minutes (to cause tail vain dilation) before restraining. Rat tail was then cleaned and massaged with methylated spirit until vein becomes visible. A 2.5G needle was used to collect blood from tail vein (about 1 to 2 ml). The blood was then left to stand for about 45 minutes before centrifuging at 10,000 x g. Serum was collected into sample bottles and stored at - $4\ ^{O}C$ until required for analysis.

Statistical Analysis.

The treatments were fitted to a Randomized Block Design (RBD). Analysis of Variance (ANOVA) was used to establish between groups. This was followed by a multiple comparison procedures (Student-Neuman-Keul test) to identify the significantly different values.

HORMONAL ASSAY

(A) Thyroid Stimulating Hormone (TSH) Assay. Thyroid Stimulating Hormone assay is based on the principle of a solid phase enzyme-linked immunosorbent assay (ELISA), Test Kit by ICN Pharmaceuticals Diagnostic Divition, Orangebury, NY 10962-1294.

(B) Total Thyroxine (T_4) Enzyme Immunoassay (EIA). The measurement of T_4 is a direct assay of a limited (competitive) type by Immunometrics (UK) Ltd, 280 Munster Road, London SW6 6BQ.

(C) Thiocyanate Assay. Simplified Colorimetric Determination of thiocyanate, method by Pittigrew A.R and Fell G.S (1972). (12).

(D) Histopathological Assessment. Rats were anesthetized with chloroform before the thyroid gland of all the groups were collected and fixed in a solution of 10% buffered

formal saline before being taken for histological examination at the Histopathology Department of the Ahmadu Bello University Teaching Hospital, Zaria.

RESULTS

As millet diet was increased to 100%, there were increases in serum TSH, with days 45 and 90 on 100% having the highest increase (Table 1). These increases were statistically significant ($p<0.001$).The levels of serum TSH also increased significantly ($p<0.05$) after 45 or more days on a fixed percentage millet diet.

Cross-sections of rat thyroid gland on 30% millet treatment show the presence of abundant normal colloid spaces and follicles on all treatment days (Plates 4a and 4b), but in the 30% treatment, the nodules show micro follicles without capsules or blood vessel invasion (Plate 4a).

Cross-section of rat thyroid gland between 60% millet treatment and control group also show the presence of normal colloid spaces on all treatment days (Plates 5a and 5b), but 60% millet treatment on day 45 indicates the presence of few pink un-nucleated akanazi cells (Plate 5a). While 60% millet treatment on day 90 show a collapse of colloid spaces highly proliferated by follicular cells and pink un-nucleated akanazi cells in the presence of enlarged capillaries (6a) when compared to control (Plate 6b).

Cross-section of rat thyroid gland between 100% millet treatment and control group on day 45 also show a collapse of colloid spaces, high proliferation of follicular cells and pink un-nucleated akanazi cells but no enlarged capillaries when compared to the control (Plate 7a and 7b).

Cross-section of rat thyroid gland between 100% millet treatment and control group on day 90 show a total collapse of colloid spaces and a germinal center with lymphocyte infiltration; a high proliferation of follicular cells with small atrophic follicles lined by Hurthle cells when compared to the control (Plates 8a and 8b).

A fixed concentration of 30% cabbage juice was added to 0%, 30% and 60% of millet diet, it was observed that the level of TSH only showed a statistically significant increase ($p<0.001$) after day 90 for groups in 30% cabbage and 30% millet (Table 2). Slight but not statistically significant were observed for other treatments (Table 2).

The histopathologial analysis of tissue cross-sections in this group where found to be normal of all treatments as in controls (Plates 4b,5b,6b,7b,8b).

A fixed concentration of 60% cabbage juice was added to 0%, 30% and 60% of millet diet. Serum TSH levels in the treatments showed slight but not statistically significant difference {($p> 0.01$) (Table 3)}.

The histopathologial analysis of tissue cross-sections in this group where found to be normal for all treatments as in controls (Plates 4b,5b,6b,7b,8b).

An increase in 100% millet diet showed significant increase in serum T_4 on day 45 and then dropped significantly on day 90, indicating a low serum T_4 level. The decrease was statistically significant ($p<0.001$) (Table 4).

A fixed concentration of 30% cabbage juice was added to 0%, 30% and 60% of millet diet. Serum T_4 levels show a statistical significant decrease only on day 45 with

30% millet and 30% cabbage juice treatment, $p< 0.001$. The slight differences observed for other treatments were not statistically significant ($p> 0.001$) Table 5.

A fixed concentration of 60% cabbage juice was added to 0%, 30% and 60% of millet diet. Differences in serum T_4 levels in all treatments were not statistically significantly enough to exclude the possibility that their slight difference was due to random sampling variability, $p> 0.03$ (Table 6).

As millet diet was increased to 100%, difference in serum thiocyanate levels in the treatments was not statistically significant enough to exclude the possibility that their slight differences was due to random sampling variability, $p> 0.01$ (Table 12).

DISCUSSION

In 1994 World Health Organization simplified goitre grading by reducing the number of goitre grades to two. However, the definition of goitre was simultaneously changed to "an enlarged thyroid that is palpable but not visible". In view of this definition, the rat thyroid glands in all treatment grouped in the experiment were neither enlarged nor palpable, thus goitre was excluded.

A major foodstuff for which goitrogenic effects have been postulated is millet. Osman (9,10), observed that rats fed pearl millet diets developed abnormal thyroid hormone patterns with hyperplasia. In this study millet and cabbage treatments similarly caused a significant increase in TSH level ($p<0.001$; Table 1) and a significantly lowered levels of T_4 ($p<0.001$; Table 4) which are indicative of hypothyroidism. Elevated serum TSH constitutes an indicator of iodine deficiency. Serum T_4 and T_3 are less specific indicators of iodine deficiency because they are modified usually only in conditions of at least moderate iodine deficiency. In moderate and severe iodine deficiency, serum T_4 is low. A biochemical picture associating elevated serum TSH in spite of normal serum T_4 and T_3 is called sub-clinical hypothyroidism while overt hypothyroidism is associated with elevated TSH and low T_4 with variable levels of T_3. Therefore the results obtained from this study tend to show a picture of overt hypothyroidism (3).

Hyperplasia is an increase in the number of the cells in a tissue. The normal thyroid follicle has a smooth lining of a single layer of follicular cells as shown in the control groups of normal thyroid gland (Plate 4b to 8b). However corresponding to groups on Plates 6a, 7a and 8a show an increase in the number of cells present in the thyroid tissues.

The thyroid gland on animals on 30% millet supplemented diet show presence of normal colloid and micro follicles in the nodules but without capsules or blood vessel invasion (Plate 4a). A similar pattern is also observed in groups on 60% millet supplemented diet but with the presence of few 'pink un-nucleated akanazi cells (Plates 5a).

Thyroid gland of rats fed with 60% millet supplemented diet on day 90 treatment and 100% supplemented diet shows a clear picture of thyroid hyperplasia (Plates 6a and 7a). These plates present a collapse of colloid cells, high proliferation of follicular cells and the presence of pink un-nucleated akanazi cells with enlarged capillaries. In addition to the above histological patterns, (Plate 8a) presents a germinal center with lymphocytes infiltrates Hurthle cells lining follicular proliferation. In this specimen, papillary

infoldings of follicular cells covering central cores of connective tissue project into the lumen of the follicles. This is similar to report of Osman (9,10) who observed the presence of abnormal thyroid hormone patterns with hyperplasia when he fed rats with millet balanced diet.

Thiocyanate results in this study showed that the mean values among treatments were not great enough to exclude the possibility that the difference is due to random sampling variability ($p>0.01$.) But while some reports have implicated thiocyanate in the pathogenesis of thyroid disorders, others have suggested that with adequate iodine intake overt hypothyroidism or goitre will not develop in the presence of high thiocyanate loads, or other goitrogens. (11).

Separate consumption of pearl millet and raw cabbage have been considered some of the factors responsible for the high incidence of goitre in some rural African communities who depend on their nutritional benefits for daily sustenance. The tag 'goitrogens' (goitre forming) have been placed on the above important African food source by scientist who have studied them as far back as 1928, when Chesney found out that rabbit fed largely on cabbage developed goitre and (9,10), and also attributed the presence of goitre found in Sudanese girls to the consumption of pearl millet from the high concentration of serum isothiocyanate he detected in their blood. But this is the first empirical evidence that simultaneous consumption of millet and cabbage as is the case in many rural countries in Africa can not synergistically act together to produce enhanced thyroid disorders since the histopathologial analysis of tissue cross-sections in these combined treatment groups where found to be normal for all treatments as in controls (Plates 4b,5b,6b,7b,8b). Thus, since millet has become a staple food grown to sustain the nutritional and economical well being of the ever-growing population of rural dwellers, in Nigeria and other African countries, and since several endemics of goitre and thyroid disorders have been attributed to dietary goitrogens acting together with iodine deficiency, it is very important that goitrogens be considered alongside iodine deficiency because of their relationship.

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RESULT TABLES AND FIGURES

Table 1: Serum TSH Levels (mIU/ml) in Rats on Millet-Supplemented Diets (X ± Sd).

TREATMENT	DAYS OF TREATMENT		
	DAY0	DAY45	DAY90
0%Millet + H_2O	0.28^{a} ± 0.18	0.51^{d} ± 0.52	0.34^{f} ± 0.34
30%Millet + H_2O	0.30^{a} ± 0.26	1.16^{c} ± 0.38	1.36^{e} ± 0.56
60%Millet + H_2O	0.30^{a} ± 0.22	2.40^{b} ± 0.93	2.14^{bcd} ± 0.42
100%Millet + H_2O	0.43^{a} ± 0.20	3.22^{a} ± 1.27	3.45^{a} ± 1.10

Data with different superscripts are statistically different (P< 0.001).

Table 2: Serum TSH Levels (mIU/ml) in Rats on 30% Cabbage Juice and Millet Supplemented Diet (X ± SD).

TREATMENT	DAYS OF TREATMENT		
	Day0	Day45	Day90
0%Millet + 30% cabbage	0.34^{a*} ± 0.22	1.28^{c*} ± 0.22	1.83^{d*} ± 0.20
30%Millet + 30% cabbage	0.33^{a} ± 0.24	1.88^{bc} ± 0.92	2.26^{b} ± 0.28
60%Millet + 30% cabbage	0.32^{a} ± 0.19	1.58^{bc} ± 0.73	1.89^{cd} ± 0.33

Data with different superscripts are statistically different (P< 0.001).

Table 3: Serum TSH Levels (mIU/ml) in Rats on 60% Cabbage Juice Supplemented Diets (X ± SD).

% TREATMENT Millet and Cabbage	DAYS OF TREATMENT		
	Day0	Day45	Day90
0%M + 60% cabbage	0.41^{a*} ± 0.32	1.97^{bc*} ± 0.78	2.34^{b*} ± 0.35
30%M + 60% cabbage	0.36^{a} ± 0.14	2.20^{b} ± 0.69	2.04^{bcd} ± 0.33
60%M + 60% cabbage	0.30^{a} ± 0.20	1.60^{bc} ± 0.41	2.20^{bc} ± 0.39

Data with different superscripts are statistically different (P< 0.001).

Table 4: Serum T_4 Levels (ng/ml) in Rats on Millet Supplemented Diets (X ± SD)

TREATMENT	DAYS OF TREATMENT		
	Day0	Day45	Day90
0%Millet + H_2O	7.66^{ab*} ± 0.2	7.07^{d*} ± 0.12	8.33^{ab*} ± 0.67
30%Millet + H_2O	7.49^{a} ± 0.13	8.25^{bc} ± 0.34	8.09^{ab} ± 0.42
60%Millet + H_2O	7.57^{b} ± 0.43	8.23^{bc} ± 0.64	8.30^{ab} ± 0.98
100%Millet + H_2O	7.50^{b} ± 0.55	9.52^{a} ± 0.07	6.24^{a} ± 0.57

Data with different superscripts are statistically different (P< 0.001).

Table 5: Serum T_4 Levels (ng/ml) in Rats on 30% Cabbage Juice Supplement Diet (X ± SD).

TREATMENT	DAYS OF TREATMENT

	Day0	Day45	Day90
0%Millet + 30% cabbage	$7.80^{ab} \pm 0.68$	$8.56^{b} \pm 0.37$	$8.63^{b} \pm 0.54$
30%Millet + 30% cabbage	$8.14^{a} \pm 0.64$	$7.24^{d} \pm 0.31$	$8.50^{ab} \pm 0.35$
60%Millet + 30% cabbage	$7.86^{ab} \pm 0.37$	$8.29^{bc} \pm 0.25$	$8.10^{ab} \pm 0.21$

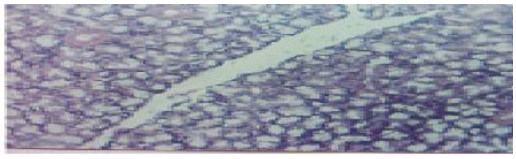
Table 6: Serum T_4 Levels (ng/ml) in Rats on 60% Cabbage Juice Supplement Diet (X ± SD)

% TREATMENT Millet and Cabbage	DAYS OF TREATMENT		
	Day0	Daay45	Day90
0%M + 60%Cabbage	$8.14^{a*} \pm 0.27$	$8.45^{b*} \pm 0.89$	$8.44^{ab*} \pm 0.89$
30%M + 60% cabbage	$7.99^{ab} \pm 0.40$	$8.19^{bc} \pm 0.17$	$8.13^{ab} \pm 0.29$
60%M + 60% cabbage	$7.86^{ab} \pm 0.40$	$8.29^{c} \pm 0.59$	$8.10^{ab} \pm 0.54$

Table 7: Serum Thiocyanate Levels (ng/ml) in Rats on Millet Supplemented Diets. (X ± SD).

TREATMENT	DAYS OF TREATMENT		
	Day0	Day45	Day90
0%Millet + H_2O	$3.19^{a*} \pm 0.23$	$3.26^{a*} \pm 0.17$	$3.17^{ab*} \pm 0.08$
30%Millet + H_2O	$3.31^{a} \pm 0.10$	$3.22^{a} \pm 0.17$	$3.17^{ab} \pm 0.08$
60%Millet + H_2O	$3.33^{a} \pm 0.07$	$3.25^{a} \pm 0.13$	$3.39^{ab} \pm 0.13$
100%Millet + H_2O	$3.33^{a} \pm 0.30$	$3.34^{a} \pm 0.17$	$3.34^{a} \pm 0.32$

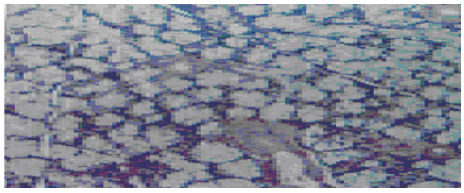
Plates 4a and 4b are cross-section of thyroid glands.



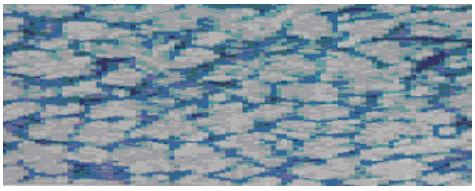
4a (30% Millet)

4b (Standard diet)

Plates 5a and 5b are cross-sections of thyroid glands on day 45.

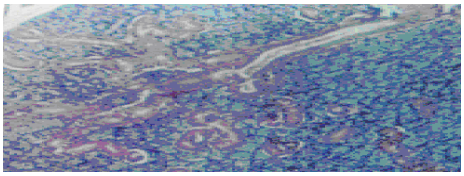


5a(60% Millet)

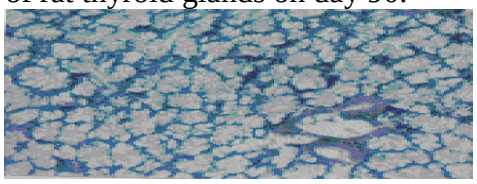


5b(Standard diet)

Plate 6a and 6b are cross-section of rat thyroid glands on day 90.

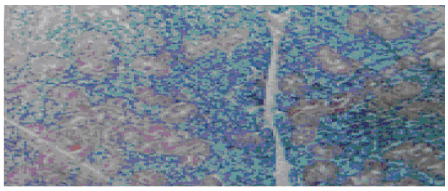


6a (60% Millet)

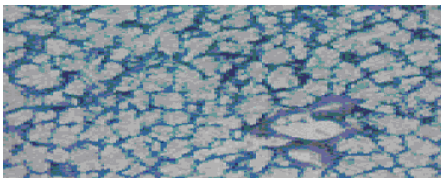


6b (Standard diet)

Plates 7a and 7b are cross-sections of rat thyroid glands on day 45.

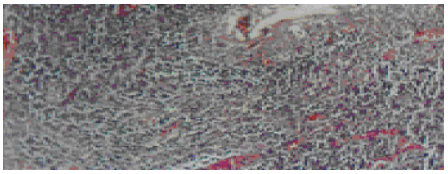


7a (100% Millet)

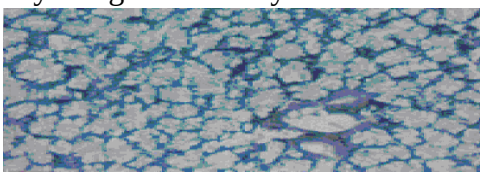


7b (Standard diet)

Plates 8a and 8b are cross-sections of thyroid glands on day 90.



8a (100% Millet)



8b (Standard diet).