ORIGINAL ARTICLE

SOYBEAN, CASSAVA AND CABBAGE LOCALLY GROWN IN A IODINE DEFICIENT AREA IN CENTRAL JAVA ARE GOITROGENIC TO RATS

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ABSTRACT

Although the relation of iodine deficiency to endemic goiter is well established, other factors may be involved. Some factors are related to food. The aim of the study was to prove that some foods locally grown in an iodine deficient area are goitrogenic. Twenty eight Wistar rats age 28 days, weight 60-70 g were randomly assigned into seven groups. The first group fed with AIN-93G as control group. Group II and III were given 25% and 50% raw soybean replacing the same weight of AIN-93G. Group IV and V were given 25% and 50% raw cassava replacing the same weight of AIN-93G. Group VI and VII were given 25% % and 50% raw cabbage replacing the same weight of AIN-93G. The length of the experiment was 34 days. The results showed that the control group fed with AIN-93G grew optimally, while the other groups showed deficits in growth significantly. Histological preparation of the thyroid gland were carried out under hematoxilyn eosin staining. Both 25% and 50% of raw soybean, raw cassava and raw cabbage produced colloid goiter. The study proved that foods locally grown in an iodine deficient area are goitrogenic.
Keywords: goitrogen, soybean, cassava, cabbage.

INTRODUCTION

Although the relation of iodine deficiency to endemic goiter is well established, other factors may be involved[1]. Some factors are related to food[2]. In 2013 our group conducted a survey in schoolchildren at Ngargoyoso sub-district, Central Java, Indonesia, concerning these factors. Using a food frequency questionnaire (FFQ) we found several foods frequently consumed in substantial amounts by schoolchildren, i.e. soybean, cassava and cabbage[3]. Soybean contains isoflavone. Consumption of soybean could produce isoflavone levels in the thyroid gland sufficient to inactivate human thyroperoxidase (TPO)[4] Cassava contains linamarin which is converted to thiocyanate after hydrolysis. Thiocyanate is the goitrogenic factor directly affects the thyroid gland[5]. Raw cabbage contains progoitrin which liberates goitrin through specific enzymatic hydrolysis by a thioglycosidase contained in plant itself[6]. Its anti-thyroid action cannot be antagonized by iodine administration[2].Since, this area has been already known as an iodine deficient area, and at present people in the sub-district only rely their iodine intake upon iodized salts, we predict their iodine status is marginal. Therefore, adding some goitrogens to their foods could be harmful. The objective of the study was to prove the goitrogenicity of foods locally grown in this area.
The study was approved by Ethical Review Committee, School of Medicine, Sebelas Maret University.

MATERIAL AND METHODS

Feeds
AIN-93G was imported from BioServ™ (Canada) in the form of half pellets. Soybean, cassava and cabbage were bought from local traditional market in Ngargoyoso sub-district, Central Java, Indonesia.

Rats
Rats of Wistar strain were inbred in the Laboratory of Nutrition, Gadjah Mada Inter University Center, Yogyakarta, Indonesia.

Subjects
Twenty eight rats age ± 28 days, weight 60-70 g were divided randomly into 7 groups and were given feeds for 34 days. Rats were given feeds as the following: Group I 100% AIN-93G (Control), Group II 75% AIN-93G + 25% raw soybean, Group III 50% AIN-93G + 50% raw soybean, Group IV 75% AIN-93G + 25% raw cassava, Group V 50% AIN-93G + 50% raw cassava, Group VI 75% AIN-93G + 25% raw cabbage and Group VII 50% + 50% raw cabbage. Feeds were given by gastric tube and distilled water was given *ad libitum*. All rats were killed with chloroform at day 35 and the thyroid glands were removed. Formalin was used as preservative before histological examination. Hematoxylin and eosin stain was used to compare thyroid follicles among groups.

Data collection
Rats were weighed before and at the end of experiments. The average of feeds consumed was

measured. Thyroid glands were prepared for histological examination with hematoxylin-eosin staining focusing on the follicles, *i.e.* its colloid and follicular cells. Light microscope with 40X enlargement was used.

Normal thyroid glands showed oval follicles with similar size. No cystic follicle. Each follicle contains colloid layered with cuboid cells.
Struma colloids showed follicles with different size. Some are cystic. Cystic follicles layered with epithelial cells. Lumen filled with colloid.
All thyroid glands were examined using the same criteria.

Statistical analysis
An independent-t test was used to compare the weight of each group with the control group. All statistical calculations were done using SPSS for Windows release 17.0 (Chicago, IL, USA). .

RESULTS

All rats were still alive at the end of the experiments. The comparison of weight before and after treatments was made (Table 1). The control group was assumed growing optimally. All experiment groups showed deficit in growth significantly, except Group II (AIN-93G + 25% raw soybean). Histological examination of the thyroid glands using hematoxylin and eosin stainshowed normal thyroid in control group (Figure 1) and colloid goiter in the experimental groups (Figures 2-4).All (28) samples of the thyroid glands were examined using the same staining and light microscope.

Table 1. Iodine intake and body weight increase of rats

Group	Iodine/kg diet	Iodine intake/day	Total iodine intake/rat	Initial body weight (g)	End body weight (g)
I	200 µg	1.56 µg	53.04 µg	64.25	109.50
II	150 µg	1.14 µg	38.76 µg	64.00	108.50[a]
III	100 µg	0.77 µg	26.18 µg	66.00	91.75[b]
IV	150 µg	1.14 µg	38.76 µg	64.50	93.75[b]
V	100 µg	0.77 µg	26.18 µg	66.00	93.00[b]
VI	150 µg	1.14 µg	40.12 µg	65.50	93.50[b]
VII	100 µg	0.77 µg	25.84 µg	65.75	85.00[b]

I = 100% AIN-93G II = 75% AIN-93G + 25% raw soybeanIII = 50% AIN-93G + 50% raw soybean
IV = 75% AIN-93G + 25% raw cassavaV = 50% AIN-93G + 50% raw cassava
VI= 75% AIN-93G + 25% raw cabbageVII=50% AIN-93G + 50% raw cabbage
[a] *statistically not significant (p>0.05)*
[b] *statistically significant (p<0.05)*

DISCUSSION

It has been known for a long time that soil factors affected nutritive value of food supply[7]. Therefore, the present study used soybean, cassava and cabbage grown locally in Ngargoyoso sub-district, an iodine deficient area of Central Java, Indonesia.

Soybean, cassava and cabbage are commonly eaten in Ngargoyoso sub-district, Central Java, Indonesia, a place known as iodine deficient area located in mountainous area. People usually consume fermented soybean known as *tempe* and *tahu*, both steamed and fried cassava and fresh or cooked cabbage from their own garden. They are also sold in traditional market cheaply[3]. Due to its goitrogenic effects we examined these foods using Wistar rats with modified technique originally from Levine *et al*[8].

Table 2. Comparison of nutrient content among feeds

Nutrient	AIN-93G	I	II	III	IV	V	VI	
Energy (Kcal/kg)	3766.0	3494.5	3323.0	3189.5	2613.0	2878.0	1990.15	
Protein (g/Kg)	178.6	211.2	166.55	134.25	89.6	133.95	89.3	
Fat (g/Kg)	70.0	90.25	72.75	52.57	35.75	52.85	35.71	
CHO (g/Kg)	643.7	558.0	432.35	569.53	408.6	498.13	352.55	
Iodine (µg/Kg)[1]	200	150	100	150	100	150	100	

[1] Assuming that raw soybean, raw cassava and raw cabbage locally grown in the study area contained no iodine.
I = 100% AIN-93G II = 75% AIN-93G + 25% raw soybeanIII = 50% AIN-93G + 50% raw soybean
IV = 75% AIN-93G + 25% raw cassavaV = 50% AIN-93G + 50% raw cassava
VI= 75% AIN-93G + 25% raw cabbageVII=50% AIN-93G + 50% raw cabbage

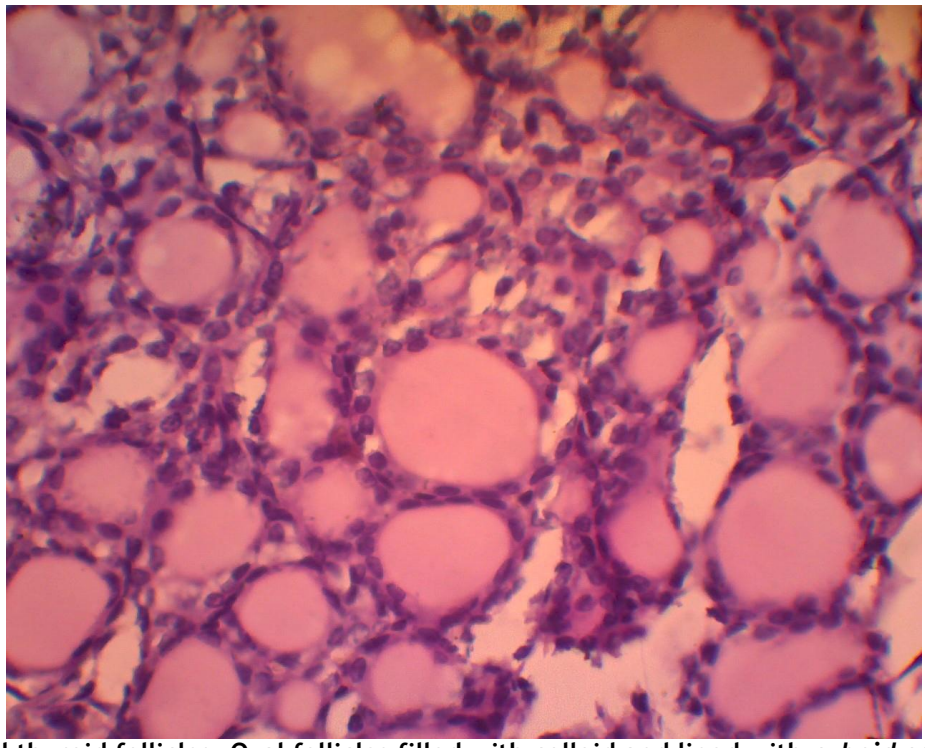

Figure 1. Normal thyroid follicles. Oval follicles filled with colloid and lined with *cuboid* cells. HE 40x magnificent.

Animal diet AIN-93G, half pellets was used as the standard diet.AIN-93G is designed for optimal growth of laboratory rodents[9]. Replacing 25% and 50% of the diet with raw soybean, raw cassava and raw cabbage would reduce energy and nutrient content as seen in Table 2. Levine *et al*[10]suggest that the iodine requirement "as the smallest amount of iodine necessary to prevent any glandular enlargement". Based on their experiments it would be 20-40 µg per 1,000 calorie of the ration. It seems that our diets are adequate in term of iodine content. Studies on goitrogenic substancesin food have been carried out since 1930's[6]. Some of them has been elucidated and confirmed by animal experiments and epidemiological data[2]. Gaitan[5] showed epidemiological data from several countries including Tasmania (milk), Finland (milk), Nigeria (cassava), Central Africa (cassava) and Sudan (millet).

Soybean is a good protein source in developing countries. In Indonesia there are two kinds of soybeans, namely imported soybean, mostly from the USA, and the locally grown. Fermented soybeans, known as *tempe* and *tahu* are very popular almost all over the country. Cassava contains linamarin which is converted to thiocyanate after hydrolysis. Thiocyanate is the goitrogenic factor directly affects the thyroid gland[5]. Combination of iodine deficiency and soy isoflavone will produce hypothyroidism[11]. The total genistein in rat serum are similar to those in humans, suggesting a similar tissue exposure, therefore it is reasonable to conclude that human isoflavone consumption could produce isoflavone levels in the thyroid gland sufficient to inactivate human TPO[11]. Indeed, it has been reported that infants with congenital hypothyroidism treated with levothyroxine and fed with soy formula continued hypothyroid. When the soy formula was

stopped but the levothyroxine was continued, the signs of hypothyroidism disappeared[13]. In our study, replacing 25% w/w of AIN-93G with raw soybean was enough to produce colloid goiter (Figure 2). We speculate that locally grown soybeans contain more isoflavone, as it is darker in color than the imported ones. Soybean powders produce goiter when it is given more than 7 weeks[14], but in our study the raw soybean can produce goiter in rats within 5 weeks.

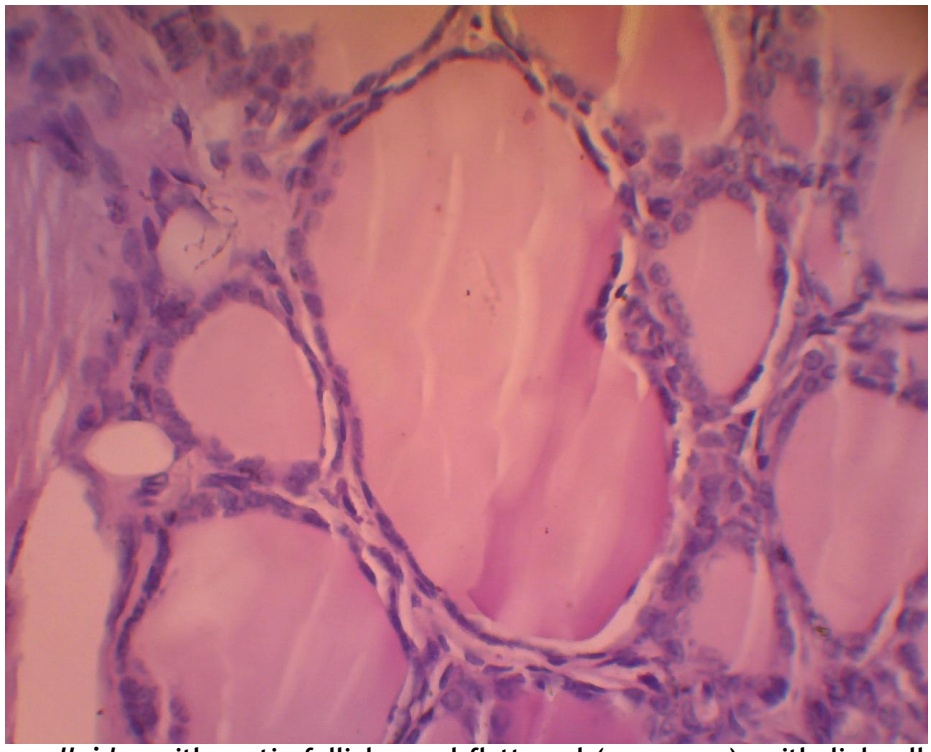

Figure 2. *Struma colloides* with cystic follicles and flattened (*squamous*) epithelial cells. After 34 days treated with raw soybean powder. HE 40x magnificent.

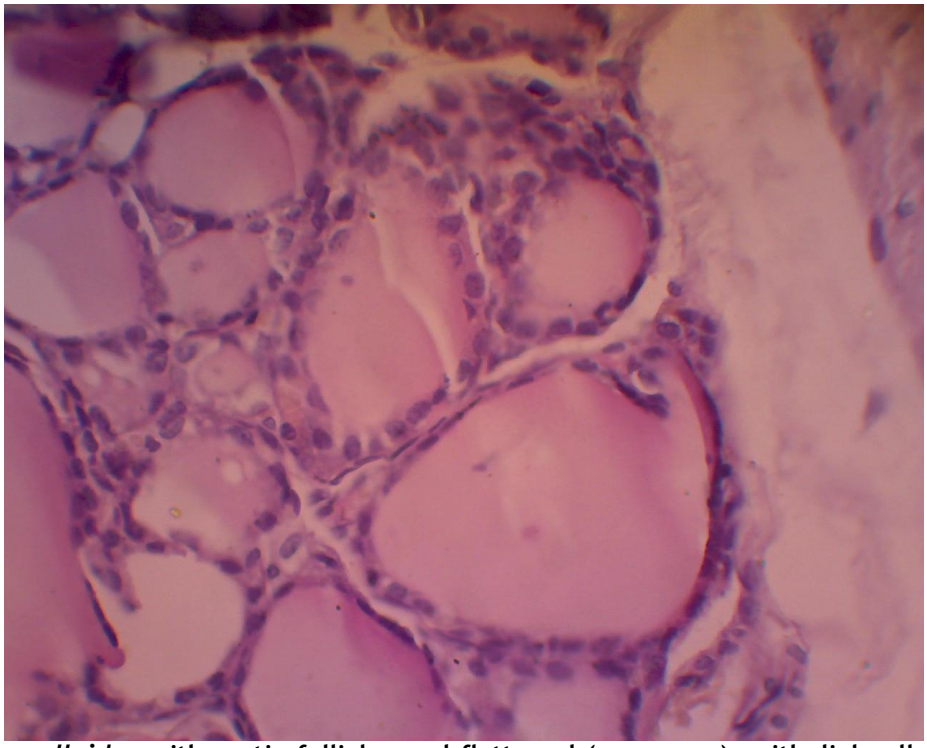

Figure 3. *Struma colloides* with cystic follicles and flattened (*squamous*) epithelial cells. After 34 days treated with raw cassava. HE 40x magnificent.

Cassava is very popular in certain areas of Indonesia. The country in 1980 produced 11.2% of the world's cassava, second only to Brazil[5]. In many parts of Indonesia cassava are the staple foods consumed daily. In Ngargoyoso sub-district, Central Java, Indonesia the staple food is rice, but cassava is consumed as snack, both steamed and deep fried. Cassava contains linamarin which is converted to thiocyanate after hydrolysis. Thiocyanate is the goitrogenic factor directly affects the thyroid gland[5]. Cassava which is grown locally in the sub-district produced colloid goiter in our Wistar rats (Figure 3). However, recent report from Thailand[12] does not support the finding.We argue there are some differences between our study and Kittivachra[12] experiment in terms of length of treatment, the standard diet and place of growing cassava. As the iodine content of the standard diet was not shown in that study, we do not know whether it is enough toovercome the effect of thiocyanate in blocking iodine uptake by thyroid gland.Goitrogenic activity of cassava can be overcome by iodine administration[2].

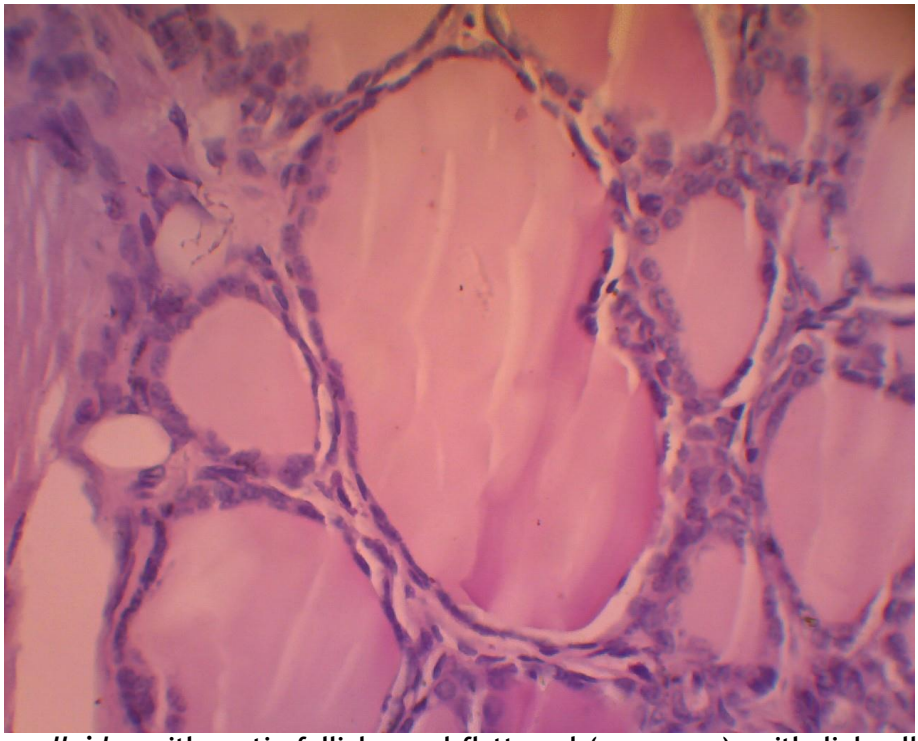

Figure 4. *Struma colloides* with cystic follicles and flattened (*squamous*) epithelial cells. After 34 days treated with raw cabbage. HE 40x magnificent.

Cabbage is the first goitrogen found incidentally by Chesney *et al*[6]. Raw cabbage contains progoitrin which liberates goitrin through specific enzymatic hydrolysis by a thioglycosidase contained in plant itself[6]. Its anti-thyroid action cannot be antagonized by iodine administration[2]. In our present study, replacing 25% w/w of AIN-93G with raw cabbage was enough to produce colloid goiter (Figure 4). In Indonesia, it is not uncommon people eat fresh cabbage with chili sauces (*sambal*).
There are two limitations of the study. Firstly, it is difficult to extrapolate the results to human being directly. However, secondly, it will be unethical to conduct such experiment in humans.

CONCLUSION

Soybean, cassava and cabbage grown locally in Ngargoyoso sub-district are goitrogenic to rats. Additive anti-thyroidal effects of soybean, cassava and cabbage in iodine deficient area as Ngargoyoso, Central Java, Indonesia could be harmful for its inhabitants.

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REFERENCES

1. Delange F ND Hetzel BS. In: Hetzel BS (eds): Toward the Global Elimination of Brain Damage Due to Iodine Deficiency. New Delhi. Oxford University Press. 2004.

2. Gaitan E. Environmental natural goitrogens. In Péter F (eds): The Thyroid

and Environment. Merck European Thyroid Symposium. Budapest 2000. June 22-25.

3. Dewi YLR. Goitrogenic foods consumed by schoolchildren in Ngargoyoso sub-district, Karanganyar regency, Central Java, Indonesia. J Nat Sci Res2013,3(2):51-56.

4. Doerge DR and Sheehan DM. Goitrogenic and Estrogenic Activity of Soy Isoflavone. Environ Health Perspect 2002, 110(suppl3):349-353.

5. Gaitan E. Epidemiological Aspects of Environmental Goitrogenesis. In: Gaitan (ed): Environmental Goitrogenesis. Florida: CRS Press, Inc. 1989.

6. Chesney AM, Clawson TA and Webster B. Endemic goiter in rabbits; incidence John Hopkins Hosp 1928, 43:261.

7. Hopkins HT, Stevenson EH and Harris PL. Soil Factors and Food Composition. Am J ClinNutr 1966, 18:390-395.

8. Levine H, Remington RE and von Kolnitz H. Studies on the Relation of Diet to Goiter: I. A Dietary Technique for the Study of Goiter in the Rat. J Nutr 1933, 4 (4): 325-345.

9. Reeves PG, Nielsen FH and Fahey GC. AIN-93 Purified Diets for Laboratory Rodents: Final Report of the American Institute of Nutrition Ad Hoc Writing Committee on the Reformulation of the AIN-76A Rodent Diet. J Nutr 1993, 123:1939-1951.

10. Levine H, Remington RE and von Kolnitz H. Studies on the Relation of Diet to Goiter: II. The Iodine Requirement of the Rat. J Nutr 1933, 4 (4): 347-354.

11. Delange F. Cassava and the Thyroid. In: Gaitan (ed): Environmental Goitrogenesis. Florida: CRS Press, Inc. 1989.

12. Kittivachra R. Effects of cassava on thyroid gland in rats. Thai J Pharm Sci 2006, 30: 57-62.

13. Fruzza AG, Berggren D and Jones KL. Unawareness of the effect of soy intake on the management of congenital hypothyroidism. Pediatrics 2012, 130 (3): 699-702.

14. Sharpless GR, Pearsons J and Prato GS. Production of goiter in rats with raw and with treated soybean flour. J Nutr 1939, 17(6):545-555.