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Effects of Geophagy on Hemoglobin Level and Iodine Absorption/ Assimilation in Albino Rats

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Abstract

Geophagic behaviour has been reported to increase exposure to Lead (Pb) that could influence the bioavailability and assimilation of iron and iodine through metal-metal interactions. Similarly, calcium that is naturally contained in kaolin tends to inhibit the absorption and assimilation of iron. In order to investigate these interactions 80 female albino rats aged 12 weeks and weighing between 212-261 g were served with Pb contaminated kaolin pellets in a manner to simulate human consumption of this clay. The size, content of the thyroid gland in iodine, urinary/faecal iodine excretion and hemoglobin level were analytically monitored during this experiment and statgraphic 5.0 used for data analyses. The results show increased thyroid volume, decreased thyroid iodine content, increased urinary iodine excretion and adequate iodine absorption as kaolin is introduced into an iodine sufficient diet. Hemoglobin levels of the various test groups compared to the control group are statistically significant. The study reveals that that kaolin-eating does not influence iodine absorption but, it influences its assimilation at the level of the thyroid gland in albino rats. The study also reveals that the consumption of kaolin affects hemoglobin level.

Keywords: Iodine; kaolin; Lead (Pb); Hemoglobin (Hb); Albino rats

Introduction

Although there exist beliefs about the nutritional benefits and detoxifying effects of geophagia [1] some reports indicate that ingestion of soil causes anemia [2-3]. Others present contrary view of no association between geophagia and anemia [4].

Geophagic behavior increases exposure to metal contaminants in the soil, including lead as reported [5-6]. Iron occupies similar niches with Pb in the human body and so competes with lead for absorption in the gut and uptake within the body [7-8]. While the primary toxicity of Pb in the body is brought about by its ability to mimic calcium it also interferes with iron metabolism in ways that are well understood [9]. The displacement of iron by zinc (Zn) in the hemoglobin structure to produce Zn protoporphyrin [7] is one of the primary consequences of lead toxicity [9], which also results to iron deficiency. Lead also reduces the production of red blood cells (erythropoiesis), their size (microcytic anemia) and their lifespan [10].

In addition, lead (Pb) is amongst the chemical factors or goitrogens that interfere with normal thyroid function and promote thyroid growth or goiter [11,12] or thyroid hormonal imbalance [13]. It may act alone or in concert with iodine-lack to produce a spectrum of clinical conditions called Iodine Deficiency Disorders (IDD) such as cretinism, physical or mental retardation, and deafness or muteness and spastic etc. [14-16].

In 1990, Lantum [17] completed a quasi-national survey in Cameroon to compile evidence establishing a national community diagnosis of total goiter prevalence of 29.4%, which is the best known sign of iodine deficiency. In 1991, Universal Salt Iodization (USI) and consumption was initiated through a ministerial order (No. 0133/A/MSP/SG/DSFM/SDSF/SN) after this baseline survey. To evaluate the impact of the USI in Cameroon the Ministry of Public Health (MPH) conducted a national survey on 30 randomly selected clusters in 2002, reporting a goiter prevalence dropping to 5.3% [18]. The etiology of this resistant goiter might not be due to iodine-lack in the diet as the incidence of goiter in animals and human subjects with normal dietary intake of iodine has been reported in controlled studies [19-22]. Lead (Pb) is known to hinder the process of iodine assimilation. Long-term low-dose exposure to lead (Pb) in sheep has been reported to affect thyroid function in hypothyroidism [23-28].

This investigation was carried out to determine the effect of kaolin-eating on body iodine status (thyroid size, thyroid iodine level, and urinary/fecal iodine excretions) as well as its influence on the Hb in albino rat.

Materials and Methods

The concentration of iodine in kitchen salt (42.5 ppm) was determined by titrimetric method [29] prior the study. Kitchen salt was uniformly mixed with the diet of the albino rats. This food was used to feed the rats starting from the time kaolin pellets were being administered.

To ensure contamination, kaolin from Nigeria, Balengou and Mbengwi were analyzed for their heavy metal load (Pb, Cd and Hg) as described [6,30] prior to the experiment. They were subsequently blended into one sample by thorough mixing so as to simulate human consumption of kaolin. It was then re-analyzed to ensure uniform heavy metals repartition.

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One hundred and twenty (120) albino rats (80 females and 40 males) aged 12 weeks and weighing between 212-261 g were used as animal model for kaolin feeding experiment and average kaolin consumption was calculated as described [6].

Diets were prepared by hand-mixing from weighed, finely powdered constituents in proportion as presented on Table 1. The basic control diet was modified by substituting 9.4% kaolin for non-nutritive bulk in group B, 22.5% for bulk in group C, 42.9% for bulk in group D and 80% for bulk in group E. The dry finely pulverized kaolin was sifted as described [6] and used for kaolin pellets for the test groups of the rats as described [5].

Two female rats were paired to one male in the rearing cages for 8 days to increase the chances of all the rats becoming pregnant. During this period vaginal smear was carried out on every rat and spermatozoids examined on the microscope to confirm pregnancy. The pregnant rats were initially weighed then divided into five groups of 16 rats. Kaolin pellets were initiated during the first day of the experiment and discontinued 21 days after parturition. Test groups B, C, and D received kaolin pellets early in the morning according to the kaolin consumption range (w/w) in human. At the same time, groups A and E were served with normal diet and kaolin above the upper consumption limit respectively. All the groups were starved overnight. Tap water was provided with rubber bottles equipped with stainless spouts.

Thyroid glands of the rats were extracted [31] from each of the five groups at days 0, 7, 14 and 21 by sacrificing the rats. They (thyroids) were weighed in comparison with the placebo. Urine and fecal samples from the different rat groups were also collected to determine iodine content on days 0, 7, 14 and 21.

In order to investigate the influence of kaolin consumption on hemoglobin, another experiment was carried out on a group of 15 albino rats (10 females and 5 males), with body weight ranging from 170-220 g. Pregnancy was ensured by pairing a male to two females in the rearing cages as described above. Kaolin was adjusted for weight every three days so as to maintain a uniform dose per body weight throughout the experiment during which the rats were divided into 5 groups and fed the same diet for 21 days as earlier described. The rats were then sacrificed at day 21 and blood collected in Ethylene Diamine Tetra Acetic Acid (EDTA) anticoagulant tubes for immediate Hb determination.

Faecal samples of rats were dried in a ventilated oven at 40°C for five days, ground to powder using a mortar and packaged in air-tight waterproof plastic papers and stored at room temperature.

The iodine contents of the thyroid glands, urine and fecal samples were determined in the various test groups and compared to the control group by alkaline dry ash methods as described [32,33].

The Centre for Research on Food and Nutrition receives, analyses and sends the result of EQUIP (Ensuring the Quality of Urinary Iodine Procedure) samples for urinary iodine from the Centre for Disease Control and Prevention (CDC) Atlanta, Georgia, USA three times a year. EQUIP is an inter-laboratory control programme for Quality Control (QC), that ensures that established urinary iodine laboratories meet the minimum expected standards. The Quality control laboratory of the Nutrition Centre (CRAN) was chosen amongst 28 laboratories in the world as an International Resource Laboratory (IRLI) to represent Central Africa, particularly francophone Africa for training, technology transfer and Quality Control (QC) purposes.

Advantage of this External Quality Assessment (EQA) was exploited by introducing EQUIP samples of known iodine concentrations as Internal Quality Controls (IQC) during iodine analyses, so as to provide accurate results. This technique helped to ensure scientific validity of the results as well as the reliability of all operations.

Hemoglobin of rat blood was determined by haemocue technique [34]. Rat blood was collected in Ethylene Diamine Tetra Acetic acid (EDTA) anticoagulant tubes and analyzed the same day in the laboratory. Each rat blood sample was analyzed in quadruple and a mean value obtained.

Statistical analyses

The data obtained were subjected to a one-way Analysis of Variance (ANOVA) according to the procedure of Steel and Torrie [35] using Statgraphic 5.0. Significantly different means were separated using the methods of Duncan [36]. The values obtained were presented as Least Significance Differences (LSD) of means at ($p<0.05$) compared to those which did not differ significantly ($p>0.05$) from the value of Duncan.

Results and Discussion

Thyroid size and iodine content

The differences in the mean weight of thyroids in grams between group A, or control group and the various test groups (B-E) are statistically significant (Table 2). The thyroid gland hypertrophies as kaolin is introduced into an iodine sufficient diet. As the kaolin quantity in the diet tends to be too high, the hypertrophic rate tends to drop, though slightly as in the case of groups D and E. Figure 1 indicates altered thyroid status and rat body weight. The thyroid as a function of percentage body weight was found to increase from group A to D, dropping slightly for group E, indicating that result was never influenced the size of the rat. The iodine contents of the thyroids on the contrary are dropping as the quantity of kaolin increases, from the control (0 g) to the various test groups as observed in Table 3 and Figure 2.

It is important to remark that iodine content of the thyroid glands is observed to drop when the thyroid hypertrophies (Figure 2). Iodine content of the glands is also observed to increase remarkably for group A

Ingredients	Percentage (%)
Dry fish	10.8
10% soya concentrate	4.3
Palm-squirrel flour	3.2
Cotton cake flour	2.1
Bone flour	2.1
0ats and barley flour	10.8
Maize flour	64.7
Multivitamin	1
Iodized salt	1.1

Table 1: Composition of rat diet.

Rat group	Mean ± STD
A	0.710 ± 0.084^{a}
B	0.858 ± 0.192^{b}
C	0.937 ± 0.211^{c}
D	0.887 ± 0.212^{b}
E	0.877 ± 0.193^{b}
Values in the same column having the same superscripts are not significantly different ($p>0.05$).	

Table 2: Evaluation of the weight of thyroid gland (in grams).

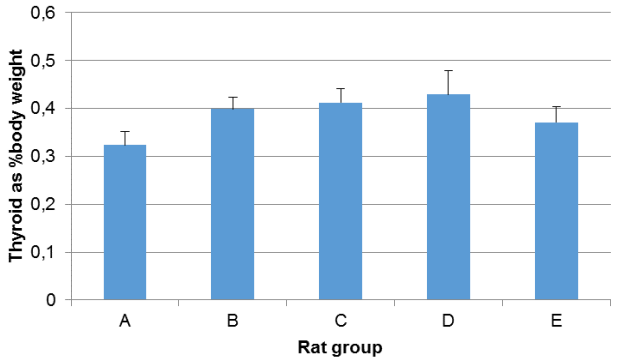

Figure 1: Thyroid as a % body weight (mean).

Rat group	Mean ± STD Iodine in µg/g of thyroid gl and/g of crude
A	690.7 ± 169[b]
B	388.9 ± 132.9[ab]
C	294.0 ± 30.9[a]
D	308.5 ± 125.1[a]
E	320.1 ± 90.1[a]
Values in the same column having the same superscripts are not significantly different (p>0.05).	

Table 3: Evaluation of the iodine content of the thyroid glands.

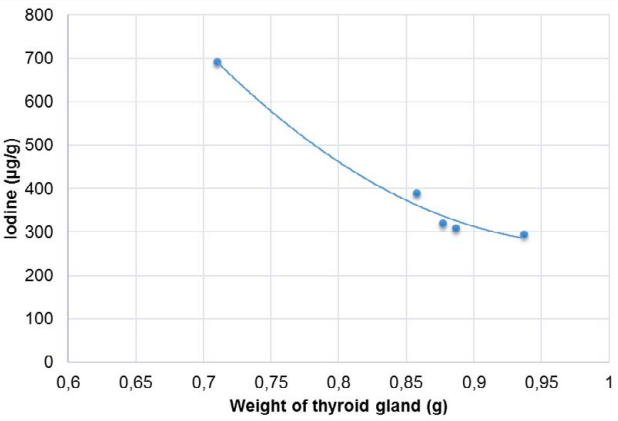

Figure 2: Thyroid iodine concentration against weight of thyroid gland.

compared to test group B (slight increased) with little kaolin intake and the rest of the test groups whose content in iodine remained virtually the same across from day 1 to day 21 (Figure 3). The control group result correlates well with the findings that thyroid glands accumulate iodine (because of increased need) to ensure adequate physiological processes during breastfeeding [37,38]. The test groups' results are indicative that kaolin affects the level of iodine in the thyroid gland. These findings are similar to those obtained by authors [39].

The incidence of goiter in animals and in human subjects with normal dietary intake of iodine has since been noticed [40], indicating that goitrogens have long been discovered. Similar effect that is not overcome by increased intake of iodine prophylaxis to the diet has been found by authors [41]. Abnormal prevalence of endemic goiter has also been observed in one locality in north-eastern Sicily after silent iodoprophylaxis from industrial products [42], though the authors suggested that this effect was due to inadequacy in the iodine prophylaxis. Furthermore, persistence or residual goiters have also been observed in post-salt iodization phase in a community with a goitrogen [43], indicating the effect of goitrogens in adequate iodine prophylaxis. In addition, authors reveal that moderate intake of iodine, adequate to meet iodine requirement, may not ensure normal functioning of thyroid in the presence of goitrogens [44], a situation that is obvious during the consumption of contaminated kaolin.

Urinary iodine level

Urinary iodine excretion (Table 4) is observed to increase gradually across from group A to E. The difference in the mean values between groups A and B or C is not statistically significant. As the quantity of kaolin increases in the diet, this difference turns to be statistically significant. Also, urinary iodine excretion is observed to increase gradually from day 1 to day 21. By day 21 the rats are either giving or have given birth and are breastfeeding. Evidence from this result corresponds to findings observed with cassava due to metabolites such as thiocyanate by Peterson [45]. Thiocyanate-like-compounds are known to primarily inhibit iodide-concentrating mechanism of thyroid gland and stimulate the iodide efflux from thyroid gland resulting to an increase in iodine excretion through urine, a situation observed in Central African Republic population [45]. Significant increase in urinary iodine excretion has also been revealed in studies involving other goitrogens in rats [44].

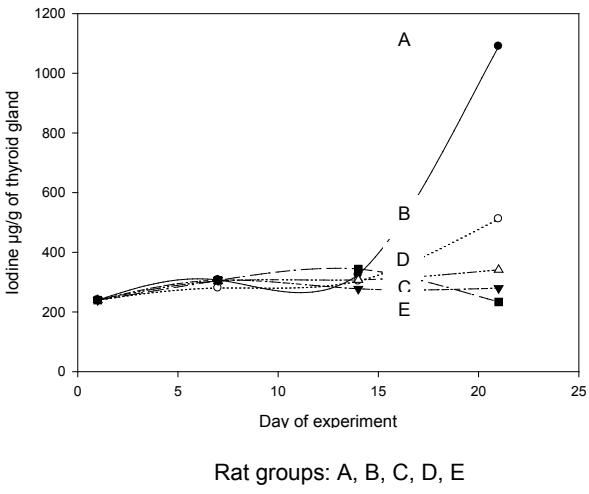

Figure 3: Iodine in the thyroid gland against day of experiment.

Rat group	Mean ± STD
A	754.7 ± 168.6[a]
B	760.7 ± 217.2[a]
C	764.7 ± 280[a]
D	956.8 ± 293.9[b]
E	919 ± 293.4[b]
Values in the same column having the same superscripts are not significantly different (p>0.05).	

Table 4: Evaluation of urinary iodine excretion (µg/l).

Faecal iodine level

Across from the control group, fecal iodine excretion (Table 5) on its part is observed to drop significantly in groups B and C, equating in group D and then increasing significantly in group E. Fecal iodine values (Figure 4) increase to day 7 and start dropping from day 14. They are lowest on day 21 correlating negatively with thyroid accumulation of iodine as mentioned above. These results do not correspond to what was obtained for cassava by Kishoyian and collaborators [46], where cassava was found to inhibit iodine absorption in the intestine. Also, a study investigating the daily intake of iodine in pigs found significant amount of iodine excreted in the feces, when their diet was supplemented with sodium humate containing 61.9% of humic acid in dry matter [22], indicating that certain goitrogenic effects occur at the intestinal level.

Exposure to Calcium (Ca) causes thyroid hyperplasia in a normal diet containing vitamin D [47]. Because kaolin contains Ca, there are chances for this effect to occur in the intestines. This indicates that the goitrogenic effect of CaO maybe amongst the effects that brought about increased kidney excretion of iodine, low thyroid concentration of iodine and thyroid hyperplasia in the albino rats (Tables 2-4).

Fecal iodine results show that iodine is absorbed in rat groups (Figure 4). Therefore, if iodine is not assimilated at the level of the thyroid gland it is not because iodine is not bioavailable. Though there is slight influence on this absorption as the quantity of kaolin consumed becomes too high (Table 5). However, no category of women was observed during field survey by questionnaire when determining consumption range in human, to consume an equivalent of 1.0 g kaolin given to this test group.

Rat group	Mean ± STD
A	628.5 ± 92.9[b]
B	513.1 ± 71.2[a]
C	501.1 ± 51.8[a]
D	615.5 ± 104.8[b]
E	711.3 ± 194.2[c]

Values in the same column having the same superscripts are not significantly different (p>0.05).

Table 5: Evaluation of faecal iodine content.

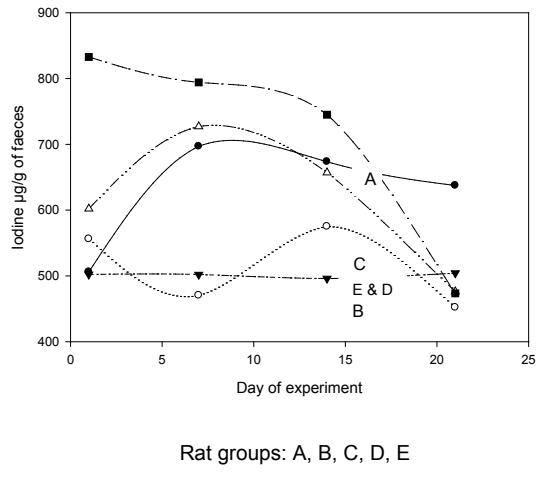

Figure 4: Faecal iodine values in the rat groups against day of experiment.

Iodine concentrations in the thyroid, urine and faeces of experimental rats were expected to be affected proportionately to the quantity of kaolin administered in the experimental groups, but the results obtained in this study did not agree with this view in all cases (Tables 2-5). Group C is a peculiar case it is observed to be highest in thyroid weight, lowest in fecal iodine with high variations in urinary iodine when we expected it to be moderate. This may be due to a common problem usually encountered in oral exposure methods. It is almost impossible to accurately determine the quantity of materials ingested by the animal; spillage and regurgitation cannot be overruled in many cases. The quantity of food and water taken by the animals also affects the amount of the material that they absorb.

The goitrogenic effect of kaolin that is observed during iodine assimilation at the level of the thyroid may also be as a result of Pb contamination. Lead (Pb) is known to affect the normal functioning of the thyroid gland. Lead causes profound endocrine manifestations in man even at levels previously considered safe [48]. Lead exposure also causes functional impairment of pituitary-adrenal axis as well as the pituitary-thyroid axis [49]. In adult sheep, poor wool growth, depressed milk yield, reduced weight gain, impaired reproductive performance with loss of libido in rams and late abortions or birth of weak lambs with visibly enlarged thyroid glands as well as an increased susceptibility to infectious agents are the most prominent clinical findings in hypothyroid cases [50]. Thus, since lead (Pb) has been observed to pass into the blood stream [5], it is one amongst the reasons for which kaolin consumption would bring about thyroid hyperplasia, low accumulation of iodine in the thyroid and high excretion of iodine in the urine of albino rats (Tables 2-4).

The sensitivity of the different organs to thyroid deficiency varies. The brain is particularly susceptible to damage during the fetal and early postnatal period [16]. This is a tragic situation to innocent neonates, (if parallel effect is true for human) who are subjected to this problem because of the kaolin consumption habit of their mothers. If the latter is the case, then the goitrogenic synergy on the thyroid gland that would be produced by CaO and Pb (a contaminant) would possibly compromise the efforts put in place by the MPH, WHO and UNICEF to ensure that adequate iodine is made available in the diet of the population so as to curb down its deficiencies.

Thyroid function is very essential to normal growth and development, especially for the foetus and hindering its function by substances like Pb and CaO will result to increase susceptibility to Iodine Deficiency Disorders (IDD). A long-term rat study with a large number of rat subjects to examine the effect of pure kaolin (consumption) on iodine absorption and assimilation can provide enough evidence (for globalization) on the goitrogenic effect of CaO. This couldn't be done in this study because of lack of pure local kaolin at the level of the market. Similarly, more investigations with thyroid effect end-points following oral exposure to lead to ensure more confidence in the results can be done alongside this experiment. Nonetheless, it is relevant to state that kaolin in the Cameroon market is a goitrogen.

Influence of kaolin consumption on Hb level

The differences in the mean hemoglobin levels in µg/dl between the control group and the various test groups (B-E) are statistically significant p=0.0002 (Table 6). The highest mean value of 14.78 ± 0.13 µg/dl occurs in group A while the lowest occurs in group C. Figure 5

Rat group	Haemoglobin (µg/dl of blood)	
	Mean ± STD	Range
A	14.78 ± 0.13 d	14.6 – 14.9
B	14.38 ± 0.17c	14.2 – 14.6
C	13.28 ± 0.22 a	13.0 – 13.5
D	13 .8 ± 0.18 b	13.6 – 14.0
E	14.25 ± 0.26 c	13.9 –14.5

Values in the same column having the same superscripts are not significantly different (p>0.05).

Table 6: Hb level of rats fed with kaolin.

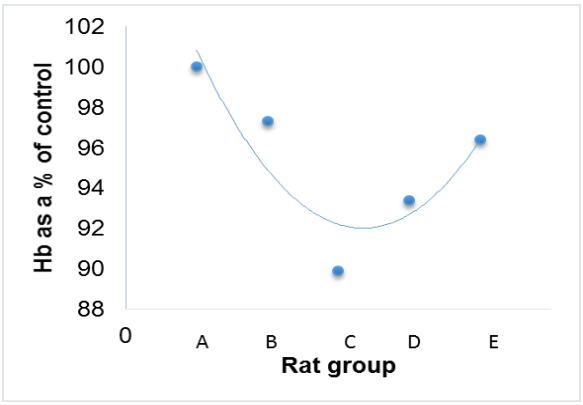

Figure 5: Haemoglobin concentration as percentage of control group value.

that illustrates the expression of Hb concentration as a percentage of the control group value shows clearly that local kaolin intake affects Hb levels in the rats.

The prevalence of anemia is defined by low hemoglobin or hematocrit level. It should be recalled that the hemoglobin cutoff used to define anemia in pregnant women living at sea level is 11 g/dl. If rat results are compared to this cutoff, then the Hb levels in the rat groups that ranged from 13.0-14.9 µg/dl were adequate certainly because the rat's diet was highly enriched with both heme iron sources (like fish, etc.) and non heme sources (like cereals, etc.). This effect of Kaolin on hemoglobin coincides to that reported [51].

However, the effect would have been different if iron was limited in the diet, since there are statistical differences in the hemoglobin levels (in µg/dl) between the means in control group and the various test groups. These findings are consistent with those of Akpantah et al. [52] that found kaolin altering the normal concentration of hemoglobin of female rats.

Conclusion

Evidence from the study reveals that kaolin does not influence iodine absorption. However, it influences its assimilation at the level of the thyroid gland in albino rats. The relationship between iodine intake and thyroid volume was found to be significant and inversely proportionate. The study also reveals that the consumption of kaolin affects Hb level.

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