EFFECT OF FISH AND SHRIMP ON IODINE BIOAVAILABILITY IN A CASSAVA AND MILLET-BASED STAPLE FOOD IN GUINEA

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Definition of Terms

Bioavailable iodine – iodine which is absorbed from food and used for the production of thyroid hormones.

Cretinism – mental and physical retardation.

Goiter – enlargement of the thyroid gland

Goitrogen – a substance that interferes with thyroid gland function, and thereby causes iodine deficiency disorders, the most spectacular form of which is goiter.

Hashimoto's thyroiditis – inflammation of the thyroid gland described by Dr. Hashimoto. It causes goiter and results in hypothyroidism.

Hormone – a chemical produced by an endocrine gland and released into the blood. It travels to other organs of the body where it produces its effect.

Hyperthyroidism – symptoms of increased metabolism due to excess thyroid hormones in the blood. It may be due to an abnormal thyroid gland, from taking thyroid medication, or from rapid increases in iodine intake in chronically deficient areas.

Hypothyroidism – symptoms of decreased metabolism due to a deficiency of thyroid hormones in the blood.

Isthmus – a small piece of thyroid tissue that connects the right and left lobes of the thyroid gland.

Myxoedema – Severe hypothyroidism.

Nodule – a lump within the thyroid gland.

Pituitary gland – a small gland the size of a peanut that is located behind the eyes at the base of the brain. It secretes hormones that control other glands (thyroid, adrenal, testicles, and ovaries) as well as affecting the kidney and growth.

Thyroglobulin – a protein in the thyroid gland, a small amount of which gets into the blood.

Thyroid binding globulin (TBG) – a protein in the blood that binds with thyroxine

Thyroid stimulating hormone (TSH) - a hormone produced by the pituitary gland that stimulates the thyroid gland.

Thyroxine (T_4) – thyroid hormone which contains four atoms of iodine known chemically as tetraiodothyronine. It is the primary hormone produced by the thyroid gland.

Triiodothyronine (T_3) – thyroid hormone containing three atoms of iodine. It is the second hormone produced by the thyroid gland. It is more potent than thyroxine.

This thesis has been written according to the format style of the Journal of Nutrition.

CHAPTER I

INTRODUCTION

Malnutrition caused by nutrient deficiency is one of the major health problems in the world. It is particularly difficult for people living in developing countries. Approximately 1.6 billion in the world are at risk for iodine deficiency alone (Cobra et al. 1997, Franke et al. 1999). Many of the people affected by iodine deficiency live in developing countries. However, some industrialized societies in Europe still suffer from iodine deficiency (Hurrell 1997, Delange 1994).

The only known role of iodine in the human body is for the synthesis of thyroid hormones (thyroxine or T_4 and triiodothyronine or T_3) which regulate a variety of physiological processes, including the metabolism rate, thermoregulation, protein synthesis, growth and development of most organs including the brain and the immune system (Cobra et al. 1997, Hurrell 1997, Stanbury 1996, Franke et al. 1999, Delange 1994). An insufficient dietary supply of iodine results in a variety of disorders grouped under the general heading of Iodine Deficiency Disorders (IDD). Among these are goiter, abortion, stillbirth, decreased cognitive function, and increased infant mortality. Iodine deficiency can hinder brain development and cause irreversible mental and physical retardation and deaf-mutism, which characterize its most dangerous form, called cretinism (Hetzel 1988, Delange 1994). Iodine deficiency may also cause cardiac insufficiency (Stanbury 1996), Kashin-Beck disease (Utiger 1998), and iodine- induced hyperthyroidism (Stanbury 1996, Stanbury et al.1998, Mann 1998). Currently there are

an estimated 655 million cases of endemic goiter and 26 million cases of preventable mental deficiency, including 5.7 million cases of cretinism worldwide (Cobra et al. 1997). Of all human beings affected by IDD, 75% are living in less developed countries (Franke et al. 1999), but 50 to 100 million people are still at risk for iodine deficiency in Europe (Delange 1994). In 1994 iodine deficiency was under control in only five European countries (Austria, Finland, Norway, Sweden and Switzerland). All other countries were still affected to varying degrees, especially in the southern and central parts of the continent (Delange 1994).

In Africa, one third of the population experiences iodine deficiency. At this point, national iodine supplementation programs and programs supported by the World Health Organization (WHO) have not succeeded in preventing related morbidity despite simple and relatively inexpensive prophylactic measures (Franke et al. 1999).

In Guinea the prevalence of iodine deficiency disorder was determined in 1994 through an epidemiological study conducted by Daffe (1994) in collaboration with UNICEF (United Nations Children's Fund). The study included 6,147 children ages 8 to 19 randomly selected throughout the country and showed a prevalence of total goiter (Grades 0 to III) equal to 63.6% with the majority having grades I and II, which indicated a prevalence of the mild form of goiter. Goiter prevalence varied in the four different natural regions that compose the country. The average rate of cretinism, mainly in its myxoedematous form, was equal to 2%. The prevalence of total goiter in Middle Guinea was 76.1%, in Forest Guinea 74.2%, in Upper Guinea 73.6% and in Lower Guinea 40.6%. The high incidence of goiter in the former three regions was partly attributed to high consumption of cassava and millet, determined by the food consumption survey that

accompanied the study, in these regions. Cassava (Delange and Ahluwalia 1983, Delange et al. 1982, Hetzel 1988) and millet (Daffe 1994, Gaitan et al. 1989, Gaitan et al. 1995) are thought to contain goitrogenic compounds that interfere with iodine uptake by the thyroid gland, and with its utilization. The relatively low incidence of goiter in Lower Guinea was thought to be caused by a relatively high intake of iodine-rich seafoods and crops in this region. Fish and seafood are the best food sources of natural iodine. By only regularly eating sea food, iodine intake can be sufficient without other measures such as salt iodization (Eckhoff and Maage 1997).

Facing such a high incidence of iodine deficiency disorders, the government of Guinea has decided to set up a plan of salt iodization, while oral iodine administrations are being distributed with the help of the WHO (World Health Organization) and UNICEF (United Nations Children's Fund). Universal salt iodization has proved to be the most efficient means of attack against iodine deficiency in many other countries. The plan was supposed to be implemented in Guinea in May 1996. So far nothing has been done because of insufficient financial means. Some iodized salts are now available in the country, but the cost of the production is entirely supported by the UNICEF. Furthermore the relatively high price of iodized salt limits its utilization in many developing countries. Actually, few countries produce iodine, and therefore its importation requires foreign currency. For example, in Mali the price of 1 kg iodized salt in 1993 was $4 while the noniodized one cost only $0.50 (Micronutrient Initiative 1996). Furthermore, the iodine content of salt even in many European countries does not reach the amount required and therefore iodine deficiency tends to persist (Burgi 1992). In addition, shelf stability is an issue for iodized salt in tropical countries (Hetzel 1988, ICCDD 1999)

Given the problems with salt iodization it is necessary to think about other alternatives or complementary measures using endogenous foods such as seafood and fish that abound in the coastal part of the country. These can contribute to the solution of the problem when efficiently used. The incorporation of seafood as an ingredient in goitrogen containing staple foods may not only increase their iodine content but also their content of some other essential nutrients such as protein, selenium, vitamin A, and iron that are also needed for normal thyroid hormone synthesis (Hetzel 1988, Cobra et al. 1997, Hurrell 1997). In Ukraine in 1992, one of the three ways of prophylaxis for iodine deficiency regulated by the government was the mandatory delivery of food allowances containing a sufficient supply of sea products to iodine deficient districts (Oleynik and Bely 1992). The transportation of fish meal in Germany once guaranteed a sufficient iodine supply for farm animals and led to a decrease in the incidence of iodine deficiency disorder in the population of the former East Germany (Anke and Groppel 1992).

Another prophylactic measure would be to reduce the goitrogens in cassava. In Zaire, Delange and coworkers (Delange and Ahluwalia 1983, Delange et al. 1982) established a detoxification process for cassava that led to an increase in iodine bioavailability in some cassava-based staple foods. The use of seafood as an ingredient in the preparation of goitrogen-containing foods in order to increase their iodine bioavailability has not been studied.

In Guinea, the forms of consumption of cassava and millet vary, but this study considered only one form: ''tô'', a porridge made of cassava and millet. This form is widely consumed in Upper Guinea, especially in rural zones where iodine deficiency disorder is more prevalent (Daffe 1994).

Objective

The major objective of this study was to evaluate fish and shrimp as iodine sources as part of a cassava and millet diet by feeding this mixture to young rats and assessing indicators of iodine status.

Hypotheses

To reach the above-mentioned objective we hypothesized that:

1- Iodine from fish or shrimp will be bioavailable from a mixture of cassava and millet.

2- Iodine status will be worsened by selenium depletion

To test these hypotheses, the following null hypotheses were considered:

$H0_1$: A diet composed of a mixture of cassava and millet will not decrease iodine bioavailability in rats compared to a diet of cornstarch.

$H0_2$: The incorporation of 6.5% of shrimp in the cassava and millet diet instead of potassium iodate (KIO_3) will not improve iodine status as much as KIO_3 does in rats.

$H0_3$: The incorporation of 6.5% of fish with low iodine in the cassava and millet diet will not improve iodine status of rats fed with this diet compared to the diet without added iodine and the diet with adequate iodine from KIO_3 or shrimp.

$H0_4$: Selenium depletion in a cassava and millet diet will not worsen iodine deficiency.

Before testing these hypotheses we found it necessary to provide the reader with some background information about the cassava-and millet-containing staple food (''tô'')

the nutritional quality of which we intend to improve, incidence of iodine deficiency and other nutritional problems in Guinea, iodine metabolism, factors controlling iodine bioavailability, requirements for iodine in humans, and indicators of iodine status.

CHAPTER II

REVIEW OF THE LITERATURE

Incidence of Iodine Deficiency in Guinea

In 1992 the German Agency for Technical Cooperation conducted a survey of 22 villages in the Southeast regions of Kissidougou and Guekedou (Forest Guinea). The survey of 3968 included children aged 8-13 and adults 18-45. A goiter prevalence ranging from 7.1 to 51.7% was found in different villages, with a median of 26.4% (Guinean Ministry of Health and Social Services 1992). Another survey in 1989 in the region of Labe, Middle Guinea, showed an average goiter prevalence of 70% affecting 58% of males and 79.1% of females. More than 50% of children in this region had a goiter, which often appeared between two to three years of age. During this survey, it was also noticed that the drinking water in this region was very low in iodine, and that the consumption of millet was high (Guinean Ministry of Health and Social Services 1992).

In 1994, Daffe conducted an epidemiological study in collaboration with UNICEF. The study included 6,147 children of ages 8 to 19 years old from all over the country. A prevalence of total goiter equal to 63.6% with a cretinism rate of 2% was found (Daffe 1994). At the same time, Konde and coworkers (1994) conducted a study involving 909 non-pregnant women (aged 18 to 40 years old) who were randomly selected from 30 districts of the prefecture of Labe, and 390 rural inhabitants (180 males and 210 females) from around the town of Labe, Middle Guinea (Konde et al. 1994). The overall prevalence of goiter among the rural inhabitants was 57% in males and

80.3% in females. There was a difference in goiter prevalence between urban women (67%) and rural women (79.5%). In the same study, the median urinary output of iodine in 106 women was 16 μg/L. Sixty nine percent of the subjects had a urinary iodine excretion below 20 μg/L, which is the value below which iodine deficiency is considered to be severe. Serum thyroid binding globulin (TBG), total T_4, and free T_4 were significantly low in stage 0, the stage at which goiter is not palpable or visible. Even in the absence of goiter, T_4 was at the lower limit of the normal value. Thyroid stimulating hormone (TSH) was at the upper limit of normal in subjects without goiter and showed a progressive rise proportionate to the thyroid swelling.

Measurement of iodine in 10 well water and stream samples in the prefecture of Labe, Middle Guinea showed a mean iodine content of 0.8 ± 0.11 μg/L (Konde et al. 1994). This amount of iodine was lower but comparable with the values in drinking water reported from areas with high incidence of goiter in Mali (4 μg/L), in two areas of Nigeria (0 to 1 μg/L), and in India (<2 μg/L) (Eckhoff and Maage 1997).

In Middle Guinea, the median urinary excretion of thiocyanide (SCN) for the 106 women was 6 mg/L with 27% of the subjects excreting more than 10 mg/L (Konde et al. 1994). In a study in Zaire (Delange et al. 1982), the mean urinary thiocyanate in the control from Brussels with adequate iodine status was 0.60 ± 0.07 mg/dL (6.0 ± 0.7 mg/L). The value in the group from the most goitrous area of Zaire with high consumption of cassava was 1.82 ± 0.10 mg/dL (18.2 ± 0.1 mg/L). The mean urinary thiocyanate in 126 pregnant women from Zaire was 2.4 ± 0.1 mg/dL. This confirms the results of the food consumption survey by Daffe (1994) which showed a relatively low

consumption of cassava (main source of thiocyanate) in Middle Guinea, but a higher consumption of millet, which is a source of other kinds of goitrogens (certain flavonoids).

Staple Foods in Guinea and their Relationship to Goiter

In Guinea there are different staple foods including rice, cassava, millet and corn. Rice is mostly eaten in urban areas, while people in the villages, where goiter is more prevalent, mostly eat cassava, millet, or corn or a mixture of cassava and millet. The food consumption survey conducted by Daffe in 1994 showed that cassava is eaten in all the four regions of the country but at different levels. In Upper Guinea 60% of families surveyed said that they eat cassava root or leaf at least once a day. This value was 50% for Forest Guinea, 20% for Lower Guinea, and 5% for Middle Guinea where a higher consumption of millet was observed (Daffe 1994)

The dish called ''tô'' is a porridge widely eaten in Upper Guinea. It is often made of cassava powder and millet. "Tô'' may also be made of the flour of only cassava, millet, maize or sorghum (Flidel 1994). A form of "tô'' made of the mixture of corn powder and corn semolina is called "kabato'' and is more popular in Siguiri, Upper Guinea and some regions of Mali and the Ivory Coast.

To make ''tô'' the millet or the semolina of corn is first boiled in water to make a gruel and then cassava powder or corn powder is gradually poured in the gruel and stirred until a homogenous thick porridge is formed. When only a flour is used, the flour is stirred into water and put on the fire for cooking. It is stirred until a homogenous, firm porridge is obtained. The porridge made exclusively of normal or fermented cassava flour and

called ``fufu'' in some other West-African countries is also a kind of ''tô''. ''Tô'' is usually eaten with an okra sauce, which may contain many other vegetables and meat or fish, at lunch, dinner, or both.

A positive correlation between cassava consumption and goiter prevalence has been established in two studies conducted by Delange et al. (Delange and Ahluwalia 1983, Delange et al. 1982) in Zaire. This study showed that chronic consumption of a large quantity of cassava did not necessarily result in the development of endemic goiter. But the development of goiter was critically related to the balance between dietary supply of iodine and thiocyanate, the latter being a goitrogen found in cassava. There is less information about the goitrogenic action of millet, but Daffe (Daffe 1994) has mentioned the presence of goitrogens in millet, and Gaitan et al. (Gaitan et al. 1989, Gaitan et al. 1995) identified in millet certain flavonoids that interfere with iodine utilization in rats. Unfortunately, these goitrogen-containing foods are widely consumed in Guinea.

Nutritional Problems and Availability of Iodine-Containing Seafood and Fish in Guinea

Guinea covers 246,000 square kilometers, which is about the area of Oregon. It is situated in the western part of the African continent and has 300 km coast with a tropical climate. The Guinean population was estimated at 5,694,296 inhabitants in 1992, unequally divided among four natural regions (Lower Guinea, Middle Guinea, Upper Guinea, and Forest Guinea). According to the Ministry of Health in 1994, more than one child out of six suffers from wasting (acute malnutrition) in the country and one out of

three suffer from growth retardation in rural areas. More than two out of ten mothers have chronic energy deficiency in Middle Guinea. Anemia affects 60 to 70% of pregnant women in the country (Guinean Ministry of Health 1994). Iodine deficiency affects 63% of children aged 8 to 19 years with a prevalence of the mild form of goiter (grades I and II) (Daffe 1994), which is thought to be sign of protein energy malnutrition (Delange et al. 1982, Delange and Ahluwalia 1983, Hetzel 1988).

Seafood and fish are the best food sources of iodine (Eckhoff and Maage 1997). The production of fish and seafood in the country in 1997 was 94,683 tons with 81,448 tons of fish (Centre National des Services Halieutiques de Boussoura 1997). This production may be increased with an increase in the utilization of modern techniques of fishing. The kind of fish called ''bonga'' (*Ethmalosa fibriata)* is very abundant and less popular, because it has a lot of small bones that make eating it inconvenient. This fish constitutes more than 30% of the yearly fish production (Centre National des Services Halieutiques de Boussoura 1997). When dried and ground, this fish may be helpful in solving the various nutritional problems the country is facing, including iodine deficiency. The kind of shrimp selected for this study (*Post-larves des penaeus durarum*) is the smallest kind of shrimp in the country. It is also relatively cheap and seems to be underused. This shrimp may also be an excellent source of iodine when efficiently used.

Iodine Metabolism and Bioavailability

Iodine occurs in food and water mainly as inorganic iodide, which is rapidly and almost completely absorbed throughout the gastrointestinal tract, including the stomach.

Iodate and protein-bound iodine in foods are reduced to iodide in the gut for absorption (Stanbury 1996, Hurrell 1997). Iodide is transported in the blood bound to plasma proteins (albumin and globulin) (Hurrell 1997). A sodium/potassium dependent active transport takes iodine into the thyroid gland, against a high gradient. In the thyroid gland, iodide is first oxidized to iodine and then incorporated into the tyrosyl residues of thyroglobulin to form mono- and diiodotyrosines (MIT and DIT). The coupling reactions of MIT and DIT lead to the formation of the complexes T_3- and T_4-thyroglobulin. Both the iodination and the coupling reactions are catalyzed by thyroid peroxidase, a heme enzyme (Hurrell 1997). A proteolytic breakdown of the iodinated thyroglobulin releases T_3 and T_4 in the blood. T_4, the principle circulating form is transformed into T_3 (the most active form of the thyroid hormone) in the liver, kidney, muscle, and thyroid under the action of a selenium dependent enzyme, type I iodothyronine deiodinase (Ruz et al. 1999). The thyroid hormone secretion is under the control of the pituitary gland through the thyroid-stimulating hormone (TSH). When plasma T_4 falls, TSH secretion is increased and thyroid activity including iodide uptake increases. In an attempt to increase iodine uptake with limited intake, TSH increases thyroid cell size and number, and the gland enlarges and forms a goiter (Hetzel 1988, Hurrell 1997)

Bioavailable iodine is that which is absorbed from food and used for the production of thyroid hormones. Other food components called goitrogens do not appear to influence iodine absorption, but they can impair its utilization for the formation of thyroid hormones (Hurrell 1997). At normal intakes, 85 to 90% of the absorbed inorganic iodine is excreted directly in the urine. The fecal excretions of iodine consist of

endogenous organic iodine (Hurrell 1997) and are considered negligible (Wahl et al. 1995).

Impact of Iodine Deficiency on Health

The synthesis and the availability of the thyroid hormones are reduced in iodine deficiency. One result of deficient thyroid hormone synthesis is the enlargement of the thyroid gland itself as it attempts to compensate for iodine deficiency (Hetzel 1988). This may not only affect the physical appearance of the patient, but also increase the risk of thyroid cancer (Delange 1994). If the thyroid nodules enlarge enough, they may also interfere with breathing and swallowing (Delange 1994).

The thyroid hormones have extensive effects throughout the body. They influence the metabolic rate, protein synthesis, enzyme function, cellular transport, thermoregulation, calorigenesis, and other physiological processes (Hetzel 1988). They have specific effects on growth in children. Throughout life a normal range of thyroid hormone is needed for active intellectual function. Low thyroid activity at crucial developmental stages causes irreversible brain damage, at its extreme expressed as cretinism and deaf-mutism (Hurrell 1997, Delange 1994, Delange 2000). Deafness, mental retardation and lowered intelligence quotient are the manifestations of lesser degrees of brain damage (Hetzel 1988). Mild to moderate intellectual impairment is an important consequence of iodine deficiency. A meta-analysis of the effect of IDD on mental development found an average of intelligence quotient (IQ) deficit of 13.5 points among iodine deficient populations (Sullivan et al. 1997).

Goiter is defined as endemic when its prevalence rate exceeds 10% in a given population. When goiter prevalence is more than 30%, 5 to 10 % of the population can be expected to have severe and irreversible mental retardation associated with anomalies of physical development known as endemic cretinism (Hetzel 1988, Delange 1994).

There are two types of cretinism, the neurological cretinism and the hypothyroid or myxoedematous cretinism (Hetzel 1988, Stanbury 1996). The clinical features of both types of cretinism are presented in **Table I.**

Table I

Comparison of the Clinical Features of Neurological and Myxoedematous or Hypothyroid Cretinism. (Adapted from Hetzel 1988)

	Neurological cretin	Myxoedematous cretin
Mental retardation	Present, often severe	Present, less severe
Deaf-mutism	Usually present	Absent
Cerebral diplegia	Often present	Absent
Stature	Usually normal	Severe growth retardation usual
General features	No physical sign of hypothyroidism	Coarse dry skin, husky voice, signs of hypothyroidism
Reflexes	Excessively brisk	Delayed relaxation
Electrocardiogram	Normal	Decreased heart rate and other abnormalities of hypothyroidism
Effect of thyroid hormones	No effect	Improvement

The neurological cretinism is a consequence of the effects of iodine deficiency and hypothyroidism in the mother during early pregnancy on fetal neural development, while hypothyroid cretinism is a consequence of iodine deficiency and hypothyroidism in infancy (Utiger 1998, Delange 1994). However, both kinds of cretinism may overlap in certain regions. The incidence of goiter is rare in both kinds of cretinism, although they happen in areas where goiter prevalence is high (Utiger 1998).

Increased fetal, prenatal, and child mortality are found with iodine deficiency and its correction is reported to increase survival (Hetzel 1988). A controlled study conducted in Indonesia in 1996 by Cobra and coworkers found that oral supplementation of infants with iodized oil may reduce infant mortality in populations at risk for iodine deficiency (Cobra et al. 1997). There are reports of an association between iodine deficiency and decreased immunity, which is reversed by administration of iodine (Cobra et al. 1997, Hetzel 1988).

Thyroid hormone deficiency seems to be a risk factor for cardiovascular disease. The effect of thyroid hormone deficiency on cardiovascular function can be characterized by decreased myocardial contractility and increased peripheral vascular resistance as well as by changes in lipid metabolism (Molnar et al. 1998). Cardiac insufficiency (Stanbury 1996, Hess et al. 1999) and high blood cholesterol and lipoprotein A (Lp(a)) (William 1991) have been observed in hypothyroid patients. Thyroid hormone administration improved the lipid profile of patients with hypercholesterolemia (Szabo and Malek 1998) and those with high levels of Lp(a) (Engler and Riesen 1993). In addition, iodine enriched eggs are commercially sold in Japan and are reported to reduce plasma levels of

cholesterol and triglycerides in laboratory animals and to increase high-density lipoprotein cholesterol in human subjects (Garber et al. 1993).

Hypothyroidism is a condition in which there are insufficient thyroid hormones in the blood. It may be caused by iodine deficiency as well as by Hashimoto's thyroiditis, inflammation of the thyroid gland described by Dr. Hashimoto, which is its most common cause in the United States (Fitz-Patrick 1996). The common symptoms of hypothyroidism are fatigue, weight gain, feeling of cold, dry skin and hair, heavy menstrual periods, constipation, and slow thinking (Fitz-Patrick 1996). It is also characterized with increased blood cholesterol and lipoprotein A (William 1991). Fertility is decreased in women with hypothyroidism, and among those who become pregnant, the frequency of preeclampsia and preterm delivery is increased. Their infants may be small for gestational age at birth and they may be slightly mentally retarded later (Utiger 1999)

Kashin-Beck disease is another iodine deficiency disorder that occurs in children and adolescents when iodine deficiency is accompanied by selenium deficiency. It is manifested as an osteoarthropathy of the hands and fingers, elbows, knees, and ankles. It is characterized by necrosis of the growth-plate and epiphyseal chondrocytes and proliferation of surrounding chondrocytes. It leads to hypertrophic osteoarthropathy and, in some subjects, to short stature (Utiger 1998).

Iodine-induced hyperthyroidism or thyrotoxicosis is another kind of iodine deficiency disorder. Iodine supplementation can aggravate thyrotoxicosis in individuals with defective thyroid glands. Iodine intake causes thyrotoxicosis only in individuals who have been exposed to years of iodine deficiency and who have, in some measure,

adapted to a low iodine intake (Wynn and Wynn 1998). Iodine induced hyperthyroidism is most commonly encountered in older persons with long standing nodular goiter and in regions of chronic iodine deficiency, but instances in the young have also been recorded (Stanbury et al. 1998). It customarily occurs after an incremental rise in mean iodine intake in the course of programs for the control or prevention of iodine deficiency. Even subclinical hyperthyroidism has health implications including disease of the cardiovascular system, arterial fibrillation, risk of thromboembolism, stroke and other embolic events (Mann 1998). The risks are primarily to older people who may have heart disease (Stanbury et al. 1998). Large amounts of iodine are tolerated by the organism in iodine sufficient areas. According to Wynn and Wynn, the iodine intake in Japan is around 1000 µg per day. A survey of 60,000 individuals in Shinsyu, Japan with a mean iodine intake of approximately 1000 µg per day found 0.2% hypothyroidism and 0.08% hyperthyroidism using serum hormone measurements (Wynn and Wynn 1998). However, an excessive intake of iodine, arbitrarily defined as 2000 µg or more per day inhibits the proteolysis and release of thyroid hormones and eventually causes iodine or toxic goiter (Delange 1994).

Factors Controlling Iodine Bioavailability

The most common cause of iodine deficiency disorders is a low content of iodine in the local environment leading to inadequate intake (Hetzel 1988). This interacts with geographical and socioeconomic factors, dietary and environmental goitrogens, some drugs (Gaitan 1990, Sauvage et al. 1999), other forms of malnutrition such as protein

energy malnutrition (Hetzel 1988), vitamin A deficiency (Horvat and Maver 1958), selenium deficiency (Hurrell 1997), and iron deficiency (Zimmermann et al. 2000a). Hormonal changes and metabolic demands during puberty and pregnancy are other causative factors of iodine deficiency disorders (Glinoer et al. 1995). Familial tendency (Abuye et al. 1999) and vegetarianism (Appleby et al. 1999) are also implicated in the etiology of iodine deficiency disorders.

Inadequacy of Iodine Intake: Geographical, and Socioeconomic Factors

Inadequate iodine intake due to environmental deficiency occurs when iodine is leached out and washed away from soil by glaciers and heavy rains in hilly and mountainous areas (Hetzel 1988). However, it is clear that IDD are significantly prevalent also in plains, and even coastal areas. Surveys in many countries of South East Asia (Hetzel 1988) and in Africa (Daffe 1994, Delange and Ahluwalia 1983) have shown the presence of IDD in most regions irrespective of their geographical conditions. Some degrees of goiter also persist in regions where iodine intake is apparently adequate (Delange 1983, Delange 1982).

Poverty and remoteness, when there is little contribution of food from outside an iodine-deficient area, as is the case with much subsistence agriculture, result in an increased incidence of iodine deficiency disorders (Hetzel 1988). Poverty, with poor sanitation and general malnutrition, may worsen the effect of iodine deficiency. Goiter incidence can decrease with socioeconomic development (Hetzel 1988). The association between IDD indicators and cognitive performance and height-for-age Z scores suggested

that socioeconomically advantaged children had better iodine status (Pardede et al. 1998). However, there is considerable evidence that iodine distribution programs have a dramatic effect as interventions, even without a rapid improvement of other aspects of poverty.

Goitrogens

Goitrogens are compounds that interfere with thyroid gland function and thereby cause IDD (Gaitan 1988, Gaitan 1990). They usually come from the environment through natural foods, food additives, water and some drugs. They may also come from some life style factors such as smoking (Delange et al. 1982, Delange et al. 1988).

Cassava is one of the few human food crops in which the content of goitrogens can cause nutritional problems (Delange and Ahluwalia 1983, Delange et al. 1982). In fact cassava contains the cyanogenic glycosides, linamarin and lotaustraline, which upon tissue damage are hydrolyzed to hydrogen cyanide. Hydrogen cyanide reacts with sulfur to form thiocyanate, which interferes with iodine uptake by the thyroid gland. The contribution of cassava to the etiology of IDD has been confirmed, especially in North Zaire (Delange and Ahluwalia 1983, Hetzel 1988). The development of goiter in the presence of a cassava–based diet depends upon the balance between dietary supplies of iodine and thiocyanate. Delange and colleagues in 1982 established critical threshold values of the I/SCN ratio related to normal conditions, endemic goiter, and hyperendemic cretinism. There is no goiter as long as the ratio of urinary iodine / thiocyanate is more

than 3 to 4. But goiter becomes hyperendemic and complicated by cretinism when the ratio of iodine to thiocyanate in urine is less than 2 (Delange et al. 1982).

According to Dunn (1993), smoking is another frequent source of thiocyanate. He states that heavy smokers (10 or more cigarettes per day) have urinary thiocyanate levels as high as those of chronic cassava eaters.

Glucosinolates, sulfur-containing glucosides yielding thiocyanates and oxazolidine-2-thiones, are found in cruciferous foods and are important constituents of crops such as cabbages, cauliflower, brussel sprouts, turnip, mustard, and broccoli (Hurrell 1997). Thiocyanates block the transport of iodine into the thyroid gland, and oxazolidine-2-thiones inhibit the iodination of thyroglobulin and the coupling of iodotyrosine residues.

Certain flavonoids capable of interfering with iodine metabolism have also been identified in millet in a study conducted at the University of Louis Pasteur of Strasbourg (Konde et al. 1994). In vivo and in vitro studies with rats led Gaitan et al. (1989) to suggest that in areas of iodine deficiency in which millet is a major component of the diet, ingestion may contribute to the genesis of endemic goiter. In fact, they identified in millet three C-glucosylflavones (glycosylvetexin, glucosylorientin, and vetexin), that inhibited thyroid peroxidase (TPO) activity. These results were strongly supported by another in vivo study with rats, in which vetexin caused a significant inhibition of TPO compared to the control (Gaitan et al. 1995).

Soybean has also been implicated in diet-induced goiter by many studies. Divi et al. (1997) observed that an acidic methanolic extract of soybean contains compounds that inhibit thyroid peroxidase-catalyzed reactions essential to thyroid hormone synthesis.

Further analyses of the extract showed that the component responsible for the inhibition of TPO co-eluted with daidzein and genistein, the major isoflavones in soybean. The Oxford Vegetarian Study, a prospective study involving 6,000 vegetarians and 5,000 nonvegetarians, conducted in the United Kingdom, showed that vegans in Britain may be at risk for iodine deficiency (Appleby et al. 1999).

A study conducted on 769 schoolchildren aged 7 to 14 in Xinjiang, Central Eurasia, suggested that a high fluoride intake exacerbated the central nervous system lesions and the somatic developmental disturbances of iodine deficiency (Fa-Fu et al. 1991). Another study in Belarus Poozerie, former USSR, showed a high incidence of goiter with low water iodine and high water manganese and nitrates (Kholodova and Fedorova 1992). This idea is shared by Horing et al. (1986) who observed impaired thyroid function in rats exposed to nitrate in drinking water. Resorcinol derivatives, which are widespread in the environment, are also involved in the etiology of IDD. Divi et al. (1997) found a significant inactivation of thyroid peroxidase and its closely related lactoperoxidase by resorcinol derivatives.

Some drugs also may show goitrogenic activity. Trimeprazine (TMP) used as an antipsychotic, and some of its main metabolites inhibited thyroid peroxidase activity and trapped molecular iodine in the thyroid gland. The administration of 5 mg twice daily to Wistar rats induced a decrease in free T_3 and T_4 and a trend toward an increase of serum thyroid stimulating hormone (TSH) (Sauvage et al. 1999).

Energy and Nutrient Deficiencies

Energy intake is a strong determinant of circulating levels of thyroid hormones. Overfeeding increases T_3 production, whereas energy restriction decreases serum T_3 concentrations (Ruz et al. 1999).

Protein energy malnutrition (PEM), vitamin A deficiency, selenium, and iron deficiencies may have secondary effects on iodine nutritional status. These deficiencies may interfere with iodine uptake by the thyroid and with thyroglobulin formation (Hetzel 1988). On the other hand, very severe PEM in some areas with extremely low iodine intake may impair the ability to form goiter with a resultant mild prevalence for this condition (Delange and Ahluwalia 1983, Hetzel 1988).

Low blood retinol levels, an indicator of vitamin A status, are correlated with higher goiter incidence (Hetzel 1988, Hurrell 1997). According to Horvat and Maver (1958), the prevalence of goiter on the island of Krk, Yugoslavia was 62% among the girls and 54 % among the boys despite a high consumption of fish. A determination of serum retinol in 80 school children revealed a severe vitamin A deficiency. The supplementation of the children with 3,000 IU per day for three months decreased goiter prevalence in 38 children forming the experimental group from 66% to 37% (Horvat and Maver 1958). Studies in Senegal showed that concomitant vitamin A deficiency increased the severity of iodine deficiency (Hetzel 1988). The suggested mechanism is that decreased retinol could reduce thyroid hormone synthesis by defective glucosylation of thyroglobulin and cause subsequent inefficient iodination. This mechanism is in line

with vitamin A's known function in controlling the production of some specific glucoproteins in tissues (Hetzel 1988).

Selenium and iodine are linked biochemically because both of them are involved in the production of thyroid hormones. Selenium is an integral component of type I 5'iodothyronine deiodinase, the enzyme responsible for the peripheral conversion of T_4 to T_3, the most potent of the thyroid hormones (Utiger 1998, Delange 2000, Ruz et al. 1999). According to Delange, the hyperstimulation of the thyroid by TSH leads to an increased production of H_2O_2 within the cells. Selenium protects the thyroid cells against oxidative damage, because it is also an integral component of glutathione peroxidase, which catalyzes the breakdown of hydrogen peroxide (Delange 2000, Utiger 1998, Ruz et al. 1999). On the other hand, selenium deficiency superimposed on iodine deficiency partly prevents the neurological damage of iodine deficiency by decreasing the conversion of T_4 to T_3 in the mother so that more thyroxine is available for transfer to the fetus (Delange 2000). However, selenium deficiency may precipitate the hypothyroid type of cretinism (Delange 2000)

Thyroid peroxidase is a heme enzyme requiring iron (Hurrel 1997). In iron deficiency, thyroid metabolism is impaired with an inability to control body temperature (Hurrel 1997). Widespread iron and vitamin A deficiency occur in developing countries, and often are seen in iodine deficient regions. In a recent study conducted in the Ivory Coast, it was suggested that the success of salt iodization in goitrous children of the study area was being hampered by anemia (Zimmermann et al. 2000a). Iron supplementation improved the efficacy of oral iodized oil in goitrous children with iron deficiency anemia (Zimmermann et al. 2000c)

Puberty and Pregnancy

The physiological stresses of puberty and pregnancy also increase the occurrence of goiter. In an epidemiological study conducted in Guinea, there was no difference in goiter prevalence between the sexes until the onset of puberty when goiter prevalence became much higher among girls than boys (Konde et al. 1994).

Pregnancy is one of the factors explaining the higher prevalence of goiter and thyroid disorders in the female population (Glinoer et al. 1995). This has been shown to be due to iodine deficiency causing damage to the thyroid during pregnancy (Wynn et al. 1998). A study conducted in Germany showed that women who had children were at greater risk of goiter and the development of thyroid nodules than women who had never been pregnant (Wynn and Wynn 1998). Iodine deficiency not only damages women but may also cause impairment of brain development of their babies (Wynn and Wynn 1998). Hormonal changes and metabolic demands during pregnancy result in profound alterations in the biochemical parameters of thyroid function. According to Glinoer et al. (1995), the main events occurring during pregnancy are:

-a marked increase in serum thyroxin binding globulin levels;
-a marginal decrease in free hormone concentrations (in iodine sufficient conditions) that is significantly amplified when there is iodine restriction or overt iodine deficiency;
-a frequent trend toward a slight increase in basal thyrotropin (TSH) values between the first trimester and term;
-a direct stimulation of the maternal thyroid gland by elevated levels of human chorionic gonadotropin (hCG), which occurs mainly near the end of the first trimester and can be associated with a transient lowering in serum TSH; and
-modifications of the peripheral metabolism of maternal thyroid hormones, including an increased turnover of maternal T_4.

These metabolic changes in pregnancy require an increased hormonal output by the maternal thyroid gland. For healthy pregnant women with iodine sufficiency, the challenge to the maternal thyroid gland is to adjust the hormonal output in order to achieve the new equilibrium state, and thereafter maintain the equilibrium until term (Glinoer et al. 1995). In contrast, this metabolic adjustment cannot be reached when the functional capacity of the thyroid gland is impaired or when pregnancy takes place in healthy women residing in areas with a deficient iodine intake. The ideal dietary allowance of iodine recommended by the International Council for the Control of Iodine Deficiency Disorders (ICCIDD) is 200 μg iodine per day for pregnant women. In conditions with iodine restriction, relative hypothyroxinemia and goitrogenesis reveal enhanced thyroidal stimulation. Goiter formed during gestation may only partially regress after parturition (Glinoer et al. 1995).

Heredity

Heredity is also one of the contributing factors for the enlargement of the thyroid gland. The familial tendency for goiter between parents and their children was found to be strongly significant in a cross-sectional study recently conducted in Ethiopia. The level of association of visible goiter was stronger between mothers and their children than between fathers and their children (Abuye et al. 1999).

Control and Prevention of Iodine Deficiency Disorders

The control and prevention of iodine deficiency are primarily based on the supplementation of salt and other foods, and the use of oral and injectable iodine or thyroid hormones. A regular consumption of naturally iodine rich foods is also recommended. Iodized salt has been so far the most efficient means utilized against iodine deficiency in many developed countries. The amount of iodine recommended by the World Health Organization (WHO) for the control and prevention of IDD is 20 to 50 mg/kg salt depending on the average daily salt intake of the population. However, the success of salt iodization is being hampered in many developing countries by many factors. These factors include salt shelf stability in tropical climates, cost of iodized salt, lack of political support, predominance of small scale salt producers making the implementation of fortification programs difficult, poor economic status, and difficulty in monitoring and evaluation (ICCIDD 1997). In addition, in 1988 most of the salt iodization units that were available in China had only one to two years of effective life in the country. This was due to rust, in spite of the plants being carefully cleaned between processing. The cost of a unit (with a capacity of 8 to 30 tons/hour) also escalated from $4,000 to $15, 000 in three years (Hetzel 1988). Furthermore in 1998 in Indonesia, 5 years after the establishment of a national salt iodization program, there still was a need to implement additional intervention measures such as the distribution of iodized oil capsules or iodizing drinking water, because most of the people in villages had limited access to iodized salt (Pardede et al. 1998).

Salt iodization is sometimes difficult to sustain causing effective programs to lapse. In 1969, 93% of salt produced in Guatemala was iodized, but this decreased to only 17% in 1976. Also in El Salvador, the production of iodized salt decreased from 56% in 1977 to 15% in 1981 (Hetzel 1988).

The utilization of intramuscular or oral administration of iodized oil has been shown to be very effective in the control of iodine deficiency. A single intramuscular injection of 4 ml of lipodiol, which provides a total of 2 g iodine, for a period of 4 to 5 years has been shown in controlled trials to decrease the incidence of cretinism in Papua New Guinea (Hetzel 1988). In 1996, a double blind, randomized, controlled trial of oral iodized oil was conducted in Indonesia to evaluate the effect of iodine supplementation on infant mortality. A total of 617 infants were allocated to receive placebo or oral iodized oil (100 mg) at 6 weeks of age and were followed to 6 months of age. There was a 72% reduction in the risk of death during the first 2 months, and a delay in the mean time to death among infants who died in the iodized oil group compared with infants who died in the placebo group (48 days versus 17.5 days). In this study oral iodized oil supplementation in infants had a stronger effect on the mortality of males compared with females (Cobra et al. 1997).

In China, iodized walnut oil and iodized soybean oil are used for oral administration (Hetzel 1988). Although severe selenium deficiency partly blunts the thyroid response to iodine supplementation, oral iodized oil was an effective method for iodine repletion in goitrous children who were selenium deficient in the western Ivory Coast (Zimmermann et al. 2000c). Unfortunately, the use of iodized oil is considered to

be useful only for short-term interventions, because it is difficult to sustain (Hetzel 1988, Sullivan et al. 1997).

The use of iodized water also has been shown to be successful in many cases. In Ohio, in 1917-22, Marine and Kimball used a dose of 200 μg of sodium iodide in water daily for 10 days in spring and repeated this in fall. They observed a satisfactory regression of goiter (Hetzel 1988). Water iodization may be more convenient than salt iodization at the village level if a specific source of drinking water can be identified, otherwise there is a heavy cost as less than 1% of a general water supply is used for drinking water (Hetzel 1988)

Sea fish and other marine foods are frequently regarded as the most important natural sources of dietary iodine. Even in inland areas, fish remain the highest natural iodine food source (Eckhoff and Maage 1997). In a study aiming to measure the iodine content of sea fish, highland fish, and different foods of plant origin in East Africa, Eckhoff and coworker found sea fish to be the highest food source of iodine. The iodine content of fish increased with the size of the fish, and iodine was more concentrated in the skin of the fish. They stated that the consumption of 150 g of medium or large changu fillet would provide nearly 150 μg iodine, the amount recommended per day for an adult (Eckhoff and Maage 1997).

In Korea, seaweed is customarily served as a soup to new mothers. Therefore, the consumption of seaweed increases drastically when a woman is lactating (Moon and Jungyeon 1999). The mean daily dietary iodine intake of 50 Korean mothers assessed by a 24-hour recall method was 2,744 μg at 2 to 5 days postpartum and decreased to 1295 μg at 4 weeks postpartum. Of this amount of iodine, 89% came from seaweed and 7%

came from milk. The colostrum and mature milk expressed at 2 to 5 days postpartum showed an average of 2,170 μg iodine/L with a wide range of 218 to 8,671 μg/L and dropped to 892 μg iodine/L at 4 weeks postpartum. The level of dietary iodine intake and the iodine content of the breast milk of Korean mothers may be much higher than in other countries, because of the higher consumption of seaweed, which is high in iodine. Their study confirms that the iodine content of breast milk reflects the maternal iodine intake. Therefore, it is thought that milk from iodine sufficient areas may also be a good source of iodine.

There are several other sources of dietary iodine. Iodate is used in the preparation of bread as a dough improver to increase the cross-linking of gluten. Bread iodization was effective in correcting iodine deficiency in the 1960s especially in Holland and Australia. A transient increase in thyrotoxicosis was observed (Hetzel 1988). In Bangkok, iodized soy sauce and iodized fish sauce are being used as additional iodized condiments (Hetzel 1988). Addition of iodide to sweets has also been used in Mexico (Hetzel 1988).

Requirements for Iodine in Humans

The recommended dietary allowances (RDA) for iodine proposed by the Food and Nutrition Board of the National Academy of Sciences of the United States in 1989 are the following: 40 μg iodine per day for infants aged zero to six months, 50 μg from six months to one year, 70 μg from one year to three years and values increasing with age up to 150 μg/day for an adult. For pregnant women and lactating women, the

recommended values are 175 µg and 200 µg per day, respectively (Food and Nutrition Board 1989). According to Delange (1993), the American Academy of Pediatrics, the European Society for Pediatric Gastroenterology and Nutrition, and the Commission of the European Communities made similar recommendations.

After critically reviewing the justification for these recommendations, Delange (1993) at the ICCIDD suggested that most of these recommendations should be increased, especially for infants, children, and pregnant women. He proposed some values that are presented in **Table II**, and compared to the ones recommended by the US National Academy of Sciences in 1989.

Table II

Dietary Allowance for Iodine as Recommended by the US National Academy of Sciences and as Proposed by the ICCIDD. (Adapted from Delange 1993)

Population group	Age (years)	US National Academy of Sciences (1989)	Proposal of the ICCIDD
		(µg/day)	
Infant	0.0-0.5	40	90
	0.5-1.0	50	90
Children	1-3	70	90
	4-6	90	90
	7-10	120	120
Adolescents/Adults	11-51 & +	150	150
Pregnant women		175	200
Lactating women		200	200

Delange (1993) states that the daily iodine requirement for the prevention of goiter due to iodine deficiency is approximately 1 μg/kg body weight, that is 50 to 70 μg/day. An allowance of 150 μg/day for adults is recommended to provide an extra margin of safety and to meet increased demands that may be imposed by natural goitrogens under certain conditions (Delange 1993). Delange also suggested that the proposal of an iodine content of formula or breast milk of 10 μg iodine/dl for full-term infants and 20 μg/dl for preterms is entirely safe as the upper limit of iodine in infant formula is 4 to 15 times higher.

Indicators of Iodine Status

Thyroid Size

Goiter, the enlargement of the thyroid gland, is a compensatory adaptation to iodine deficiency. The pituitary gland responds to low levels of circulating thyroid hormones by increasing the secretion of its hormone, thyroid stimulating hormone (TSH), which drives the thyroid to enlarge, to increase iodine uptake from the blood and to produce more hormones. Other conditions may also cause goiter, but these rarely reach a prevalence over 5% in a iodine sufficient population (ICCIDD 1999). Precisely assessed thyroid size is one of the most sensitive indicators of community iodine nutrition (ICCIDD 1999). It is usually determined by palpation or by ultrasonography, the latter being more precise. Thyroid size reflects iodine nutrition over months or years. Thyroid size is classified from Grade 0 to 5. Grade 0 corresponds to a thyroid not palpable or visible; Grade 1, the isthmus of the thyroid palpable; Grade 2, both lobes of the thyroid

palpable; Grade 3, thyroid visibly enlarged; Grade 4, large goiter with deformity of the neck; Grade 5, a giant goiter (Gerasimov 1993).

Urinary Iodine

Urinary iodine concentration is currently the most practical biochemical marker for iodine nutrition (ICCIDD 1999). Most iodine absorbed in the body eventually appears in the urine, therefore urinary iodine is a good marker of very recent dietary intake. In individuals, urinary iodine excretion can vary from day to day and even within a given day, but this variation tends to damp out in populations. In general, 30 urine determinations from a given sampling group are sufficient to evaluate urinary iodine (ICCIDD 1999). Many analytical techniques for determining urinary iodine exist. Most methods depend on iodine's role as catalyst in the reduction of ceric ammonium sulfate (yellow color) to the cerous form (colorless) in the presence of arsenious acid (the Sandell-Kolthoff reaction) (Dunn 1993, ICCIDD 1999).

Blood Levels of Thyroid Stimulating Hormone (TSH) in Neonates

A blood spot of TSH in neonates is a valuable indicator of iodine nutrition. In iodine sufficient populations, about 1 in 4,000 neonates has congenital hypothyroidism usually from inadequate thyroid development (ICCIDD 1999). Prompt correction with thyroid hormone is essential to avoid permanent mental retardation. Thyroid hormone affects proper development of the central nervous system, particularly its myelination, a

process that is active in the perinatal period. This so-called transient neonatal hypothyroidism is more frequent in iodine deficient communities, because the neonatal thyroid has limited iodine stores, and even mild deficiency may increase TSH secretion. Thus the incidence of transient elevation of TSH in neonates is a valuable indicator of community iodine nutrition (ICCIDD 1999).

Blood Levels of Thyroglobulin

Thyroglobulin is the most abundant protein of the thyroid, providing the matrix for thyroid hormones. Normally, small amounts are secreted or leak from the thyroid into the circulation. When the thyroid is hyperplastic or injured, much larger amounts are released. The thyroid hyperplasia of iodine deficiency is regularly associated with increased serum thyroglobulin. In this setting, serum thyroglobulin reflects iodine nutrition over months or years, in contrast to urinary iodine concentration, which assesses more immediate iodine intake.

Blood Levels of Thyroid Hormones

Determining serum concentrations of the thyroid hormones T_4 and T_3 is usually not recommended for monitoring iodine nutrition because these tests are more cumbersome, more expensive, and less sensitive as indicators (ICCIDD 1999). The prevalence indicators of IDD and criteria for a significant public health problem are presented in **Table III** (adapted from Delange 1994).

To determine whether the supplementation of a cassava and millet diet with fish or shrimp will improve iodine status compared with an iodine-deficient diet, or improve iodine status as much as diets supplemented with adequate amounts of potassium iodate in rats, proximate analysis of samples of cassava, millet, fish, and shrimp from Guinea was done and we determined the iodine content of these same foods. Then we considered an experimental design that allowed us to determine and compare the iodine status of rats fed with 6 different diets. We also looked at some indicators of health to see whether the supplementation may have adverse effects on the general health status of the animals

Table III

Prevalence Indicators of IDD and Ccriteria for a Significant Public Health Problem. (Adapted from Delange 1994)

Variables	Normal	Mild	Moderate	Severe
Prevalence of goiter in school-age children (SAC) (%)	<5	5-19.9	20-29.9	>30
Frequency of thyroid volume in SAC >97th percentile by ultrasound (%)	<5	5-19.9	20-29.9	>30
Median urinary iodine in SAC and adults (ug/L)	100-200	50-99	20-49	<20
Frequency of neonatal TSH >5 uU/ml in whole blood (%)	<3	3-19.9	20-39.9	>40

CHAPTER III

METHODOLOGY

Sampling of Foods from Guinea

Samples of cassava (*Manihot esculenta or utilissima*) powder and millet (*Dactylaria exilis*) were randomly taken in different markets of the town of Kankan, Upper Guinea, where cassava and millet are mostly eaten in the form of "tô". Samples were collected on July 23 and 24, 1999. The millet was polished, washed and sundried for eight hours and kept in plastic bags.

The seaweed (*Sessivumy portulacastrum*) was collected from the sea in Conakry on July 10, 1999. This seaweed was first dried at room temperature (22.7 – 28.2 °C) for three days and then in an oven at 100 °C for 3 hours. Then it was ground and kept in plastic bags.

The samples of dried fish (*Ethmalosa fibriata*) and shrimp (*Post-larves des penaeus durarum*) were bought in different markets of Conakry on July 15 and 16, 1999. Since it had been raining continuously for one week at purchase time (rain fall in July in Conakry is 11,346 mm) and the relative humidity of the air was high (97% with an average temperature varying between 22.7 and 28.2°C), it was necessary to dry the fish and shrimp again in an oven at 100^0 C for 3 hours. Then the samples were ground using a manually operated mill and kept in plastic bags. All the samples were transported from

Guinea to the laboratory of the Department of Nutritional Sciences at Oklahoma State University in Stillwater, Oklahoma.

Proximate Analysis of the Food Sample

Samples were analyzed by the Analytical Services Lab of Kansas State University. They assessed the dry matter, the amount of crude protein, crude fat, crude fiber and the calories per gram (cal/g) in the samples. The carbohydrate (CHO) content of the samples was calculated by subtracting the sum of the contents of the above-cited nutrients from the total amount of dry matter. The results of this analysis were necessary for making the diets for the rats, because we had to correct for the difference of fat, protein, and fiber among the 6 diets.

Determination of Iodine Content of the Food and Urine Samples

For the determination of iodine content of the food samples, the method proposed by Dunn et al. (1993) was used. The method depends on the role of iodide as a catalyst in the reduction of the ceric ion (Ce^{4+}) to the cerous ion (Ce^{3+}) coupled to the oxidation of As^{3+} to As^{5+}. The ceric ion has a yellow color while cerous ion is colorless. Thus the course of the reaction can be followed by the disappearance of yellow color as the ceric ion is reduced. With other reactants held stable, the speed of this color disappearance is directly proportional to the amount of iodine catalyzing it. The principle and the procedure for the assessment of iodine in food samples and in the urine of the rats were

the same. However, to get a liquid extract of the food samples, 0.5 g of each cassava, millet, fish, and seaweed were mixed with 2.5 ml deionized water in 13 x 100 mm test tubes, left to stand overnight, vortexed, and centrifuged. An aliquot of 250 µl of the supernatant was used for the determination. For the sample of shrimp, 0.25 g was mixed with 7.5 ml deionized water, and treated as the above-cited samples to get 250 µl of the supernatant. For the sample of salt, 0.1 g was mixed with 2.5 ml deionized water, left overnight, and vortexed. Thereafter, an aliquot (100 µl) of this solution was mixed with 4 ml of deionized water. Then a 250 µl amount of the latter solution was used for the analysis. To prepare aliquots of rat's urine, the frozen urine samples were thawed and centrifuged before taking the 250 µl sample for the analysis. The preparation of reagents and the test procedure are described in **Appendix A.**

Animal Experiment

Study Design

To test the research questions 1 through 4, 50 male weanling Sprague Dawley rats (21 days old) ordered from Harlan Teklad (Indianapolis, Indiana) were randomly assigned to one of the six diets presented in **Table IV** for ad libitum feeding for 5 weeks.

The first question compares the iodine status of rats on CornsKIO_3 to the iodine status of those on CMKIO_3 in order to see whether cassava and millet really contain goitrogens that affect the parameters of iodine status measured. The second hypothesis compares the iodine status of rats on CMshrimp to the iodine status of those on CMKIO_3

and CornsKIO$_3$, to see whether supplementation with 6.5% shrimp will be equivalent to KIO$_3$ as a source of iodine. The third hypothesis compares the iodine status of rats on CMfish and the iodine status of those on CM-I, to see whether fish containing a small amount of iodine may help to maintain adequate iodine status when consuming a cassava, millet, and soy diet.

Table IV
Study Design

Diet	Treatment	n	Iodine status
CornsKIO$_3$ (Control)	Cornstarch/soy protein/potassium iodate	8	Adequate iodine
CMKIO$_3$	Cassava, millet/soy protein/ potassium iodate	8	Adequate iodine
CMshrimp	Cassava, millet/soy protein/shrimp	9	Adequate iodine
CMfish	Cassava, millet/soy protein/fish	9	Low iodine
CM-I	Cassava, millet/soy protein/no added iodine	8	Iodine deficient
CM-I-Se	Cassava, millet/soy protein/no added iodine no added selenium	8	Iodine and selenium deficient

The fourth hypothesis compares the iodine status of rats on CM-I to those on CM-I-Se in order to see whether combined iodine and selenium deficiencies further impair iodine status.

The amount of iodine provided by the AIN 93 diet for growing rodents (AIN G 93) is 0.2 mg per kg diet (Reeves et al. 1993). Using 65 g of shrimp per kg diet (6.5% of the diet) provided this amount of iodine. The fish we used was very low in iodine, so the addition of 65 g per kg diet provided 0.029 mg iodine per kg diet, which is only about

15% of the recommended value. The compositions of each of the six different diets are presented in **Table V**. Soy protein (from Protein Technologies International, Saint Louis, Missouri), soybean oil, and fiber (cellulose) were added to all of the diets, but at different levels to correct for the differences in protein, fat and fiber contents of the fish, shrimp, cassava, and millet. Equal amounts of vitamin and mineral mix recommended for growing rodents were added to all of the six diets with the exception of the specified omission of iodine in diets 3 through 6 and selenium in diet 6. The compositions of the vitamin mix and mineral mix used are presented in **Appendix B** and **C** respectively.

Animal Feeding and Handling

All the rats were weighed on the day of their arrival to determine their initial body weight. Their initial weight varied between 38.2 and 50.8 g. During the feeding period, the rats were individually housed and fed ad libitum. They had free access to deionized water, which was changed twice per week. The rats were weighed twice per week to monitor their rate of weight gain. The leftover and wasted diets were also weighed twice per week to ascertain whether they should be given more feed and to calculate their daily iodine intake from their daily feed consumption.

After five weeks of experimental diets, just before necropsy, the rats were put in metabolic cages at 7:30 pm for a 12-hour urine collection. During this time they were deprived of food but had access to deionized water. A 12-hour urine sample was collected from each rat at 7:30 am the following day. Rats were anesthetized with an intraperitoneal injection of ketamine (75 mg/kg body weight) and xylazine

Table V

Composition of the Experimental Diets (g/kg diet)

Components	Control	$CMKIO_3$	CMshrimp	CMfish	CM-I	CM-I-Se
Carbohydrate						
Total	699.5	607.7	587.7	598.6	607.7	607.7
Cornstarch	699.5	20	-	11	20	20
Cassava	-	466.4	466.4	466.4	466.4	466.4
Millet	-	121	121.2	121.2	121.2	121.2
Protein						
Total	150	150.4	149.9	150.2	150.4	150.4
Soy protein	150	130	89	78	130	130
Shrimp	-	-	40.4	-	-	-
Fish	-	-	-	51.8	-	-
Cassava	-	10.4	10.4	10.4	10.4	10.4
Millet	-	10.0	10.0	10.0	10.0	10.0
Fat						
Total	50	50	50.2	50.2	50	50
Soybean oil	50	46	45	42.0	46	46
Shrimp	-	-	1.2	-	-	-
Fish	-	-	-	4.2	-	-
Cassava	-	2.8	2.8	2.8	2.8	2.8
Millet	-	1.2	1.2	1.2	1.2	1.2
Vitamin mix	10	10	10	10	10	10
Mineral mix w. I	35	35	-	-	-	-
Mineral mix-I	-	-	35	35	35	-
Mineral mix-I-Se	-	-	-	-	-	35
Iodine						
Total	0.20	0.20	0.21	0.029	-	-
KIO_3	0.20	0.20	-	-	-	-
Shrimp	-	-	0.21	-	-	-
Fish	-	-	-	0.029	-	-
Fiber						
Total	50.0	50.1	50.1	49.6	50.1	50.1
Cellulose	50.0	44.0	41.0	43.5	44.0	44.0
Cassava	-	6.1	6.1	6.1	6.1	6.1
Shrimp	-	-	3.0	-	-	-
L-cystein	3	-	-	-	-	-
L-methionine	2.5	-	-	-	-	-

KIO_3 = potassium iodate. CM = cassava and millet. fish = low iodine from fish. shrimp = adequate iodine from shrimp. -I = without added iodine. -I-Se = without added iodine and selenium. Mineral mix w. I = mineral mix recommended by AIN G 93 (Reeves et al 1993). Mineral mix-I = Mineral mix recommended by AIN G 93 without potassium iodate. Mineral mix-I-Se = Mineral mix recommended by AIN G 93 without potassium iodate and sodium selenate.

(10 mg/kg body weight). The rats were weighed while they were sleeping to determine their final body weights. After that, their blood was drawn from the abdominal artery before they died. Blood was transferred into a test tube and centrifuged to obtain serum, which was kept in a freezer (-20 °C) for later analyses. At the same time, the rats were dissected to collect the thyroid gland, liver, kidneys and spleen. The liver was trimmed of adhering tissue, weighed, and divided into three portions, which were also stored in the freezer (-20° C) for use in future projects. The thyroid gland, the kidneys, and the spleen were also trimmed, weighed and discarded. To make sure we were actually getting the thyroid gland, six thyroid samples were sent to Oklahoma Animal Disease Diagnostic Laboratory for histological analysis. The result of this analysis presented in **Appendix D** confirmed the presence of thyroid gland in all the samples they examined. The use of rats in this study was approved by the Institutional Animal Care and Use Committee Action (see the signed approval form in **Appendix E**).

Assessment of Serum Thyroxine of the Rats

The assessment of serum thyroxine was done using a radioimmunoassay kit called Coat-A-Count Total T_4 (Diagnostic Products Corporation). It is a solid phase ^{125}I radioimmunoessay, designed for the quantitative measurement of total circulating thyroxine in serum and plasma.

The method is a solid phase radioimmunoassay based on antibody-coated tubes and human serum calibrators. ^{125}I-labeled T_4 competes for a fixed time with T_4 in the sample for antibody sites, in the presence of blocking agents for thyroid hormone-binding

proteins. After the tubes are decanted and counted, the T_4 concentration is read from a calibration curve. The components of the kit and the test procedure are in **Appendix F.**

Assessment of Urinary and Serum Thiocyanate of the Rats

The method of König modified by Lundquist et al. was used to determine thiocyanate in serum and urine (Lundquist et al. 1995). Thiocyanate is adsorbed on a weak anion-exchange, and eluted with perchlorate. Thiocyanate is then chlorinated by hypochlorite and quantified by the use of isonicotinic acid and 1,3-dimethyl-barbituric acid. The preparation of the reagents and the test procedure are described in **Appendix G.**

Statistical Analyses

Since we had an unbalanced design (a design with an unequal number of subjects per group), we used the General Linear Model procedure (PROC GLM) in SAS (Statistical Analysis System) (SAS Institute Inc 1998). The general linear model is an analysis of variance suited for designs that are unbalanced (Cody and Smith 1997). This method allowed us to determine the effects of the different diets on the parameters of concern. The value of a parameter was considered to be significantly different between any two diets when the P value was less than 0.05.

CHAPTER IV

RESULTS AND DISCUSSION

Proximate Analysis of the Food Samples

The results of the proximate analysis of the food samples, presented in **Table VI**, show that cassava and millet are mainly composed of carbohydrate and that fish and shrimp are mainly composed of protein. Millet is much higher in protein than cassava. Shrimp contains some fiber while the fiber content of fish is negligible. Cassava and millet are very low in fat but fish and shrimp do have some fat.

Table VI
Proximate analysis of the food samples

Samples	% Dry matter	% Crude protein[a]	% Crude fat[a]	% Crude fiber[a]	% CHO[a]	Cal/g
Cassava flour	85.99	1.84	0.50	1.08	82.57	3297.26
Millet	88.29	6.69	0.78	0.00*	80.82	2370.51
Shrimp	89.48	62.20	1.86	4.63	20.79	3507.65
Fish	91.37	79.69	6.49	0.00*	5.19	4715.79

*Zero or below the detection levels of the method used
CHO = Carbohydrate (by difference method)
a = As-is basis

Determination of Iodine Content of the Food Samples

These results presented in **Table VII** indicate that shrimp is far richer in iodine than the kind of fish we analyzed, and that the seaweed we measured contained less iodine than the fish. The sample of iodized salt obtained from the Ministry of Health in Guinea contains more than four times the upper limit of iodine recommended by the World Health Organization (WHO) for the fortification of salt (50 µg/g). The iodine concentration of cassava, millet, soy protein, and okra were less than the detection limit of the method used.

Table VII
Iodine content of the food samples

Samples	Iodine contents (mg/kg)
Shrimp	3.29 ± 0.17
Fish	0.44 ±0.02
Seaweed	0.24 ± 0.02
Cassava	0.00*
Millet	0.00*
Okra	0.00*
Iodized salt	203.24 ± 34.19
Soy protein	0.00*

*Zero or below the detection levels
Values are means and standard deviations of ten replicate essays for each sample

Daily Iodine Intake of the Rats

The estimated daily iodine intake of the rats, calculated from feed consumption, is presented in **Appendix H** and **Figure 1.** These data showed no significant differences in the mean daily iodine intake of the rats on adequate iodine either from KIO_3 or from shrimp, but the values for $CMKIO_3$ and CMshrimp tended to be higher than that of the control. The mean daily iodine intake for the group on fish with low iodine was significantly lower than the three groups with adequate iodine, but was significantly higher than the values for groups on iodine-depleted diets (CM-I and CM-I-Se).

Effect of the Diet on Weight Gain, Weight Gain Pattern, and Organ Weight.

The results of the effect of the diets on weight gain are presented in **Appendix H** and best illustrated in **Figure 2.** The effect of the diets on weight gain pattern is presented in **Figure 3.** Rats in the group on cassava and millet with low iodine from fish (CMfish) had the highest weight gain (138 ± 5 g) followed by the rats in the group consuming cassava and millet with adequate iodine from shrimp (CMshrimp) (128 ± 5 g). The lowest weight gain was observed in the control group that consumed cornstarch with adequate iodine from KIO_3 ($CornsKIO_3$ or control) (113 ± 5 g). This value was significantly lower than those observed in CMfish and CMshrimp. There was not a significant difference between the group consuming $CornsKIO_3$, and the groups fed with cassava and millet with adequate iodine from KIO_3 ($CMKIO_3$) (122 ± 5 g), cassava and

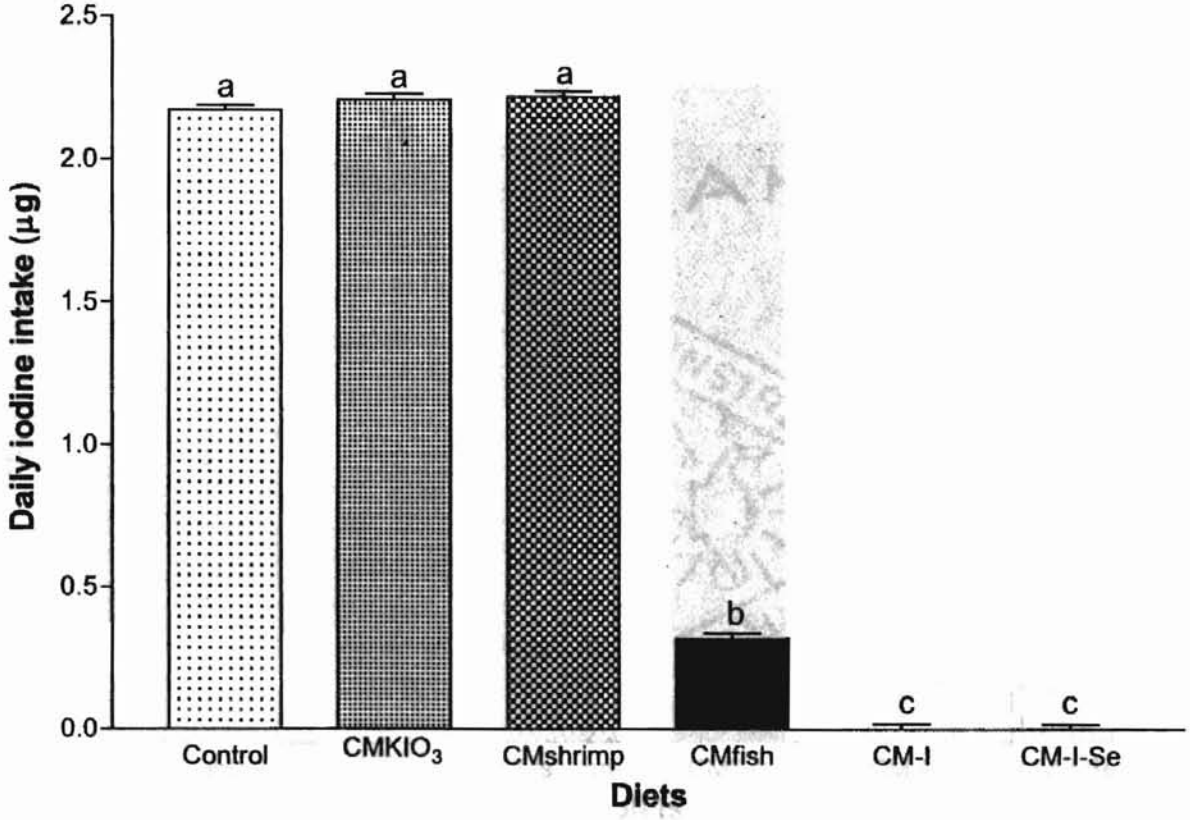

Figure 1: Daily iodine intake of the rats

Bars with different letters are significantly different ($P<0.05$)

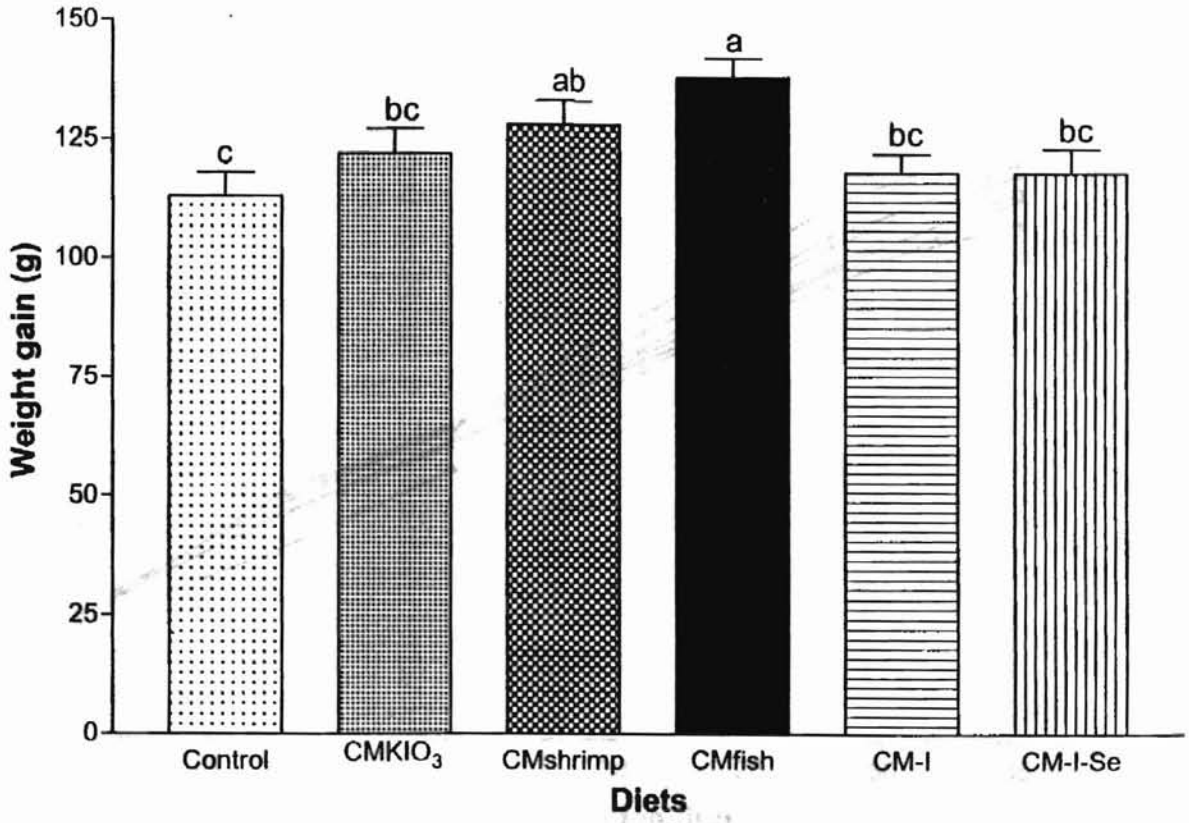

Figure 2: Effect of the diets on weight gain

Bars with different letters are significantly different (P<0.05)

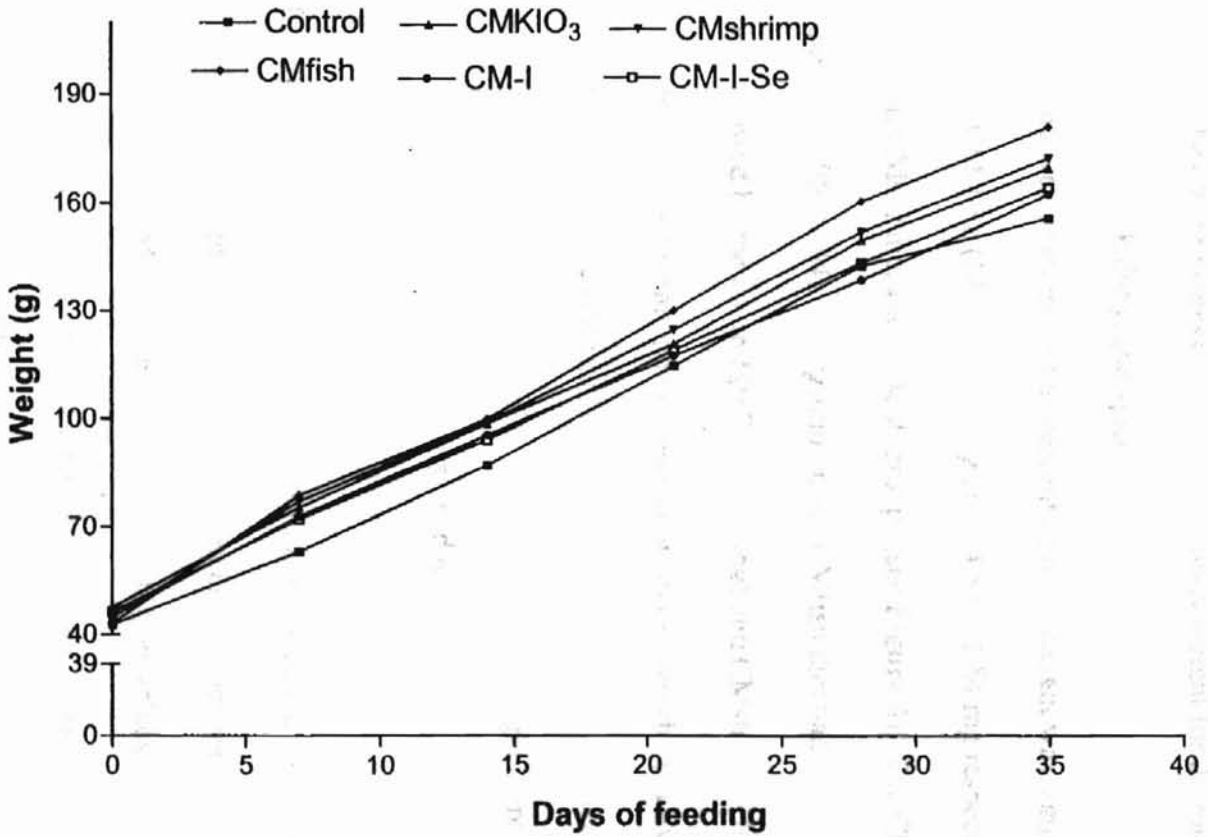

Figure 3: Effect of the diets on weight gain pattern

millet without added iodine (CM-I) (118 ± 5g), and cassava and millet without added iodine and selenium (CM-I-Se) (118 ± 5 g).

Weights of thyroid, and the other organs (liver, spleen, and kidney) are presented in **Appendix H.** Because organ weight may also vary as a function of body weight, they were expressed as mg/100g body weight for thyroid and as percent body weight for liver, kidney, and spleen.

Thyroid weight expressed as percent of body weight was significantly higher in the CM-I and CM-I-Se (14.82 ± 1.54 mg and 14.87 ± 1.54 mg respectively) groups than in the control, CMshrimp, and CMfish groups (see **Figure 4**). The $CMKIO_3$ group was intermediate (11.95 ±1.44). The group on the CMfish diet had the lowest value (8.60 ± 1.36 mg) followed by the control (9.74 ± 1.44 mg) and CMshrimp (10.03 ± 1.44 mg).

There was not a significant difference in spleen weight and liver weight as percent of body weight among the animals fed the six diets. Liver weight was higher in CMfish, but when liver weight was expressed as percent of body weight there was not a significant difference among the six diets. Kidney weight was not significantly different among the control, $CMKIO_3$, CMfish and CMshrimp. Rats on these four diets had significantly higher kidney weight than rats in the groups fed with CM-I and CM-I-Se. But when kidney weight was expressed as a percent of body weight, only the control and CMshrimp had significantly higher values. On a percent body weight basis there was no significant differences among $CMKIO_3$, CMfish, CM-I, and CM-I-Se for the kidney (see **Figure 5**).

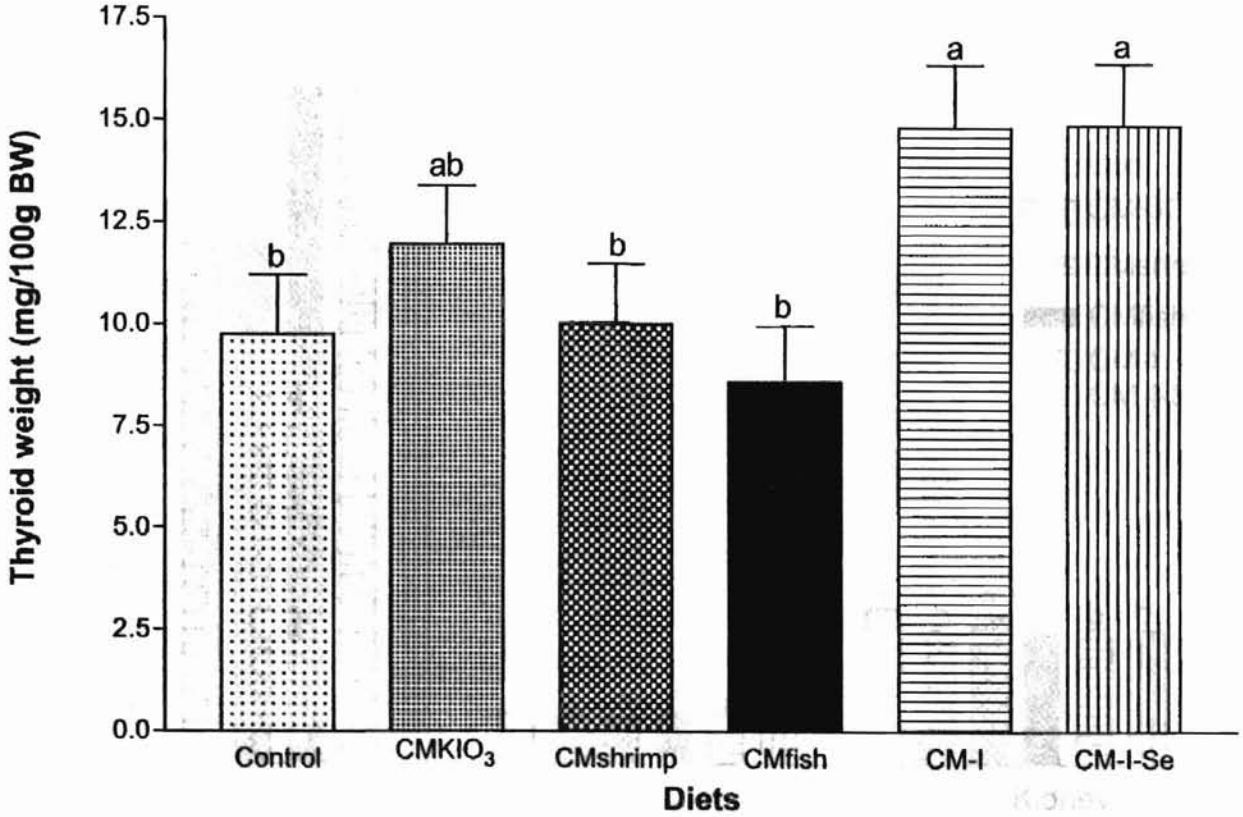

Figure 4: Effect of the diets on thyroid weight as mg/100g body weight

Bars with different letters are significantly different ($P<0.05$)

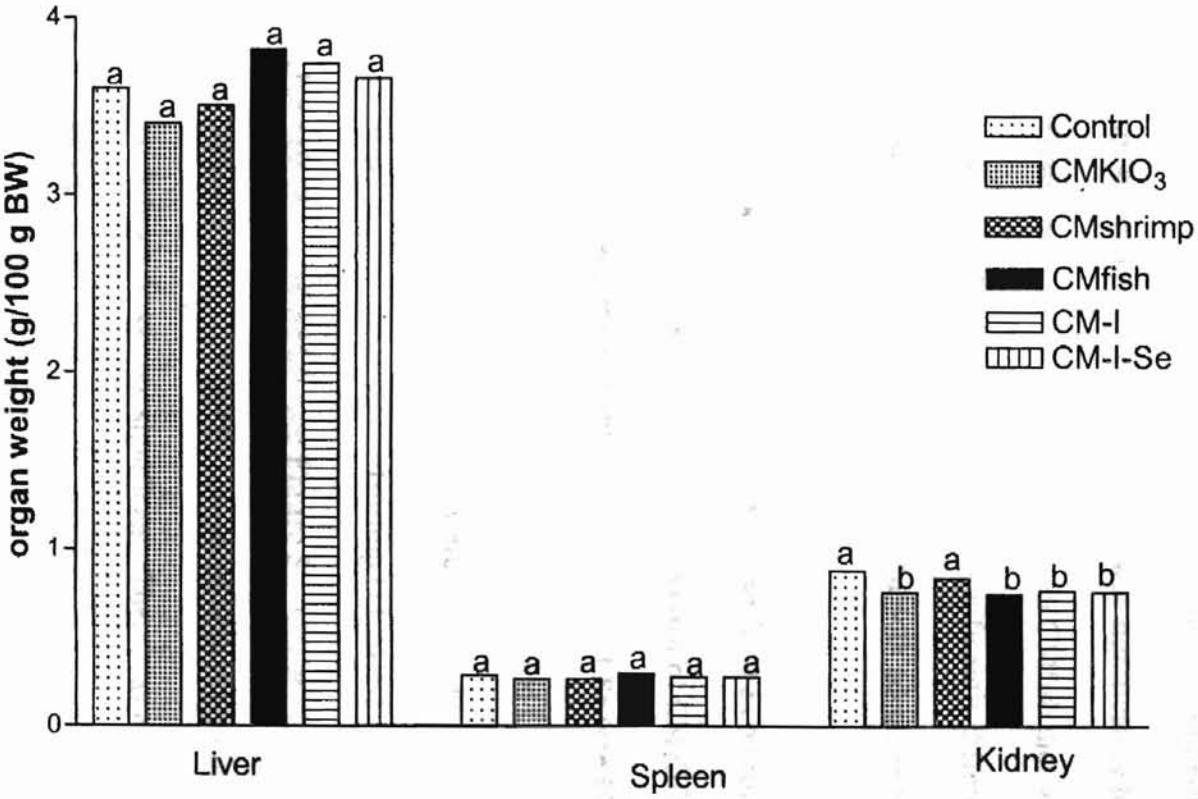

Figure 5: Effect of the diets on liver, spleen, and kidney weights

Bars with different letters for the same organ are significantly different ($P<0.05$)

Effect of the Diets on Serum Thyroxine, Serum Thiocyanate, Urinary Iodine, and Urinary Thiocyanate of the Rats.

The results of the effect of the diets on serum thyroxine, serum thiocyanate, urinary iodine, and urinary thiocyanate, are presented in **Appendix I.**

Serum thyroxine in the control group was significantly higher than in any other group (see **Figure 6**). The value for rats fed with CMfish diet was significantly higher than that of CM-I and CM-I-Se, and was not significantly different from the values for the groups fed cassava and millet-supplemented with adequate iodine from KIO_3 or shrimp.

Serum thiocyanate was significantly lower in the control than any other group. The value for CM-I was significantly lower than that of $CMKIO_3$, CMshrimp, and CMfish, but was not signifcantly different from CM-I-Se (see **Figure 7**). There were no significant differences among $CMKIO_3$, CMshrimp, and Cmfish, and CM-I-Se.

The highest urinary iodine values were observed in the group fed $CMKIO_3$ diet (11.42 ± 1.14 µg/dl), the group fed CMshrimp (11.24 ± 1.14 µg/dl) and the control group, CornsKIO_3 (10.14 ± 1.14 µg/dl) (see **Figure 8**). Urinary iodine tended to be lower in the group fed CMfish (8.73 ± 1.07 µg/dl) compared with the groups fed with adequate iodine, but the difference was not significant. There was not a significant difference between $CMKIO_3$, CMshrimp, CMfish and the control (CornsKIO_3). Urinary iodine values in the groups fed CM-I and CM-I-Se diets were significantly lower than those observed in the control, $CMKIO_3$, CMshrimp, and CMfish groups. The difference

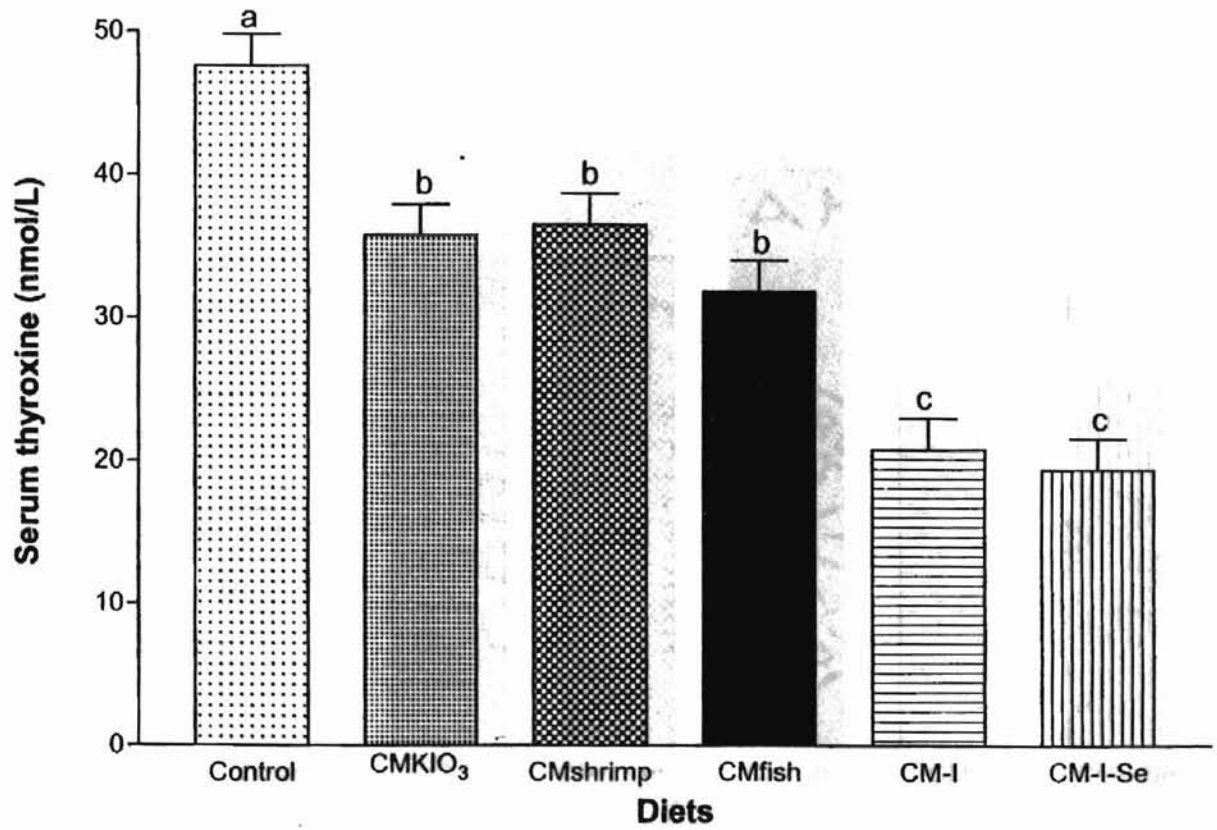

Figure 6: Effect of the diets on serum thyroxine

Bars with different letters are significantly different ($P<0.05$)

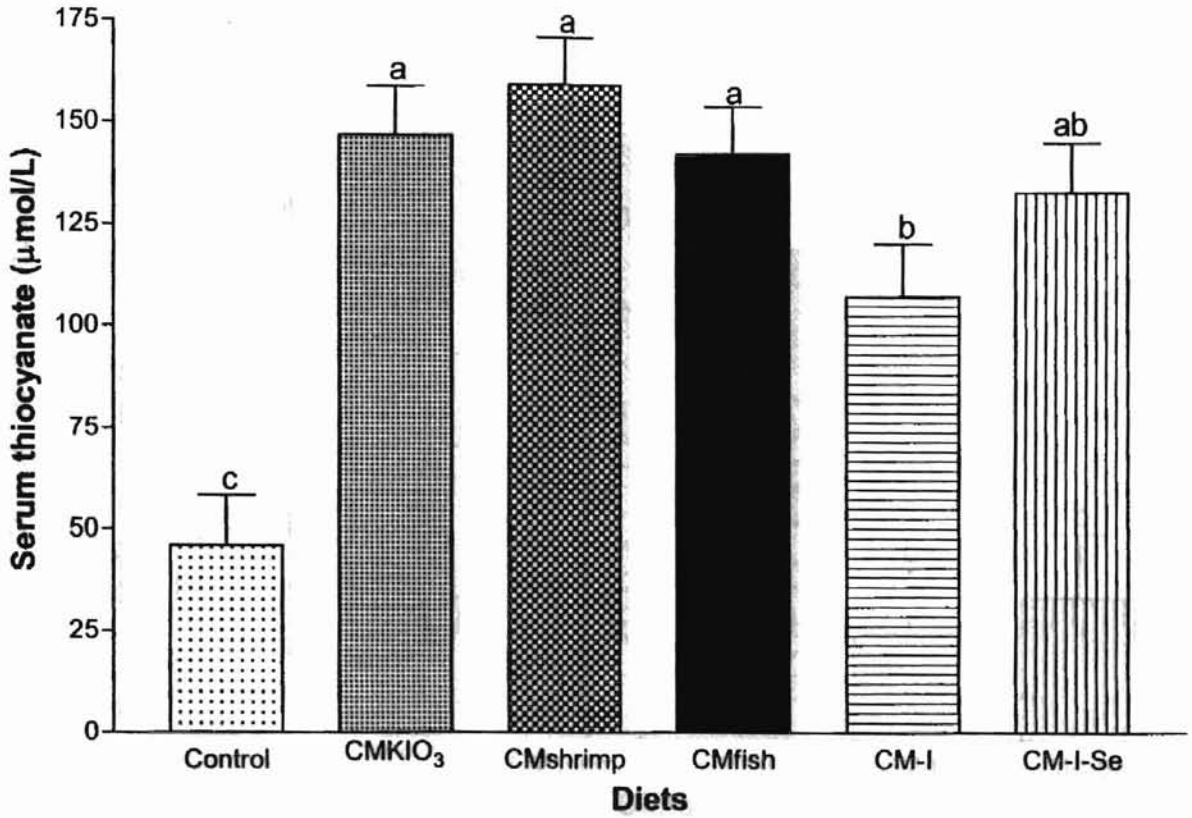

Figure 7: Effect of the diets on serum thiocyanate

Bars with different letters are significantly different ($P<0.05$)

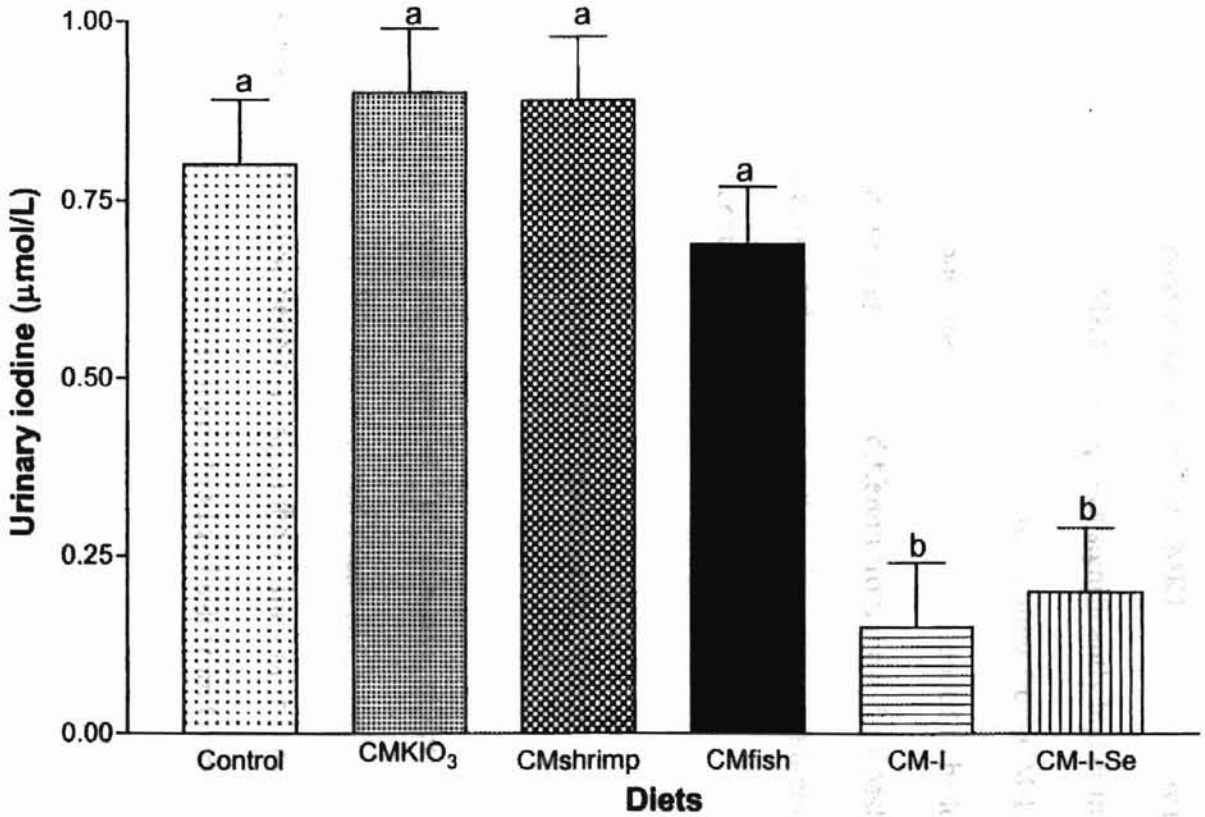

Figure 8: Effect of the diets on urinary iodine

Bars with different letters are significantly different ($P<0.05$)

between CM-I group (2.64 ±1.14 µg/dl) and CM-I-Se group (1.85 ± 1.14) was not significant

The mean urinary thiocyanate was significantly lower in the control than in any group on a diet containing cassava and millet (see **Figure 9**). The highest urinary thiocyanate was observed in the group fed $CMKIO_3$ followed by the iodine-depleted groups. The value for $CMKIO_3$ group was significantly higher than that for CMfish group, but was not significantly different from values from CM-I, CM-I-Se, and CMshrimp diets, although the value for CMshrimp tended to be lower. The value for CMfish group was not significantly different from those for CMshrimp, CM-I, and CM-I-Se. The concentration of thiocyanate in the urine was much higher than in the serum for all the groups, except for the CMshrimp group.

Discussion

Each of the main ingredients of "tô" (cassava, millet, and okra) were analyzed for their iodine content. We also analyzed a seaweed, which is abundant on the Guinean coast and eaten by some people living in the vicinity of the sea. Fish and shrimp intended for use as supplements and a sample of iodized salt from Guinea were also analyzed for their iodine contents.

The iodine content of fish was about 15% that of the shrimp. This low iodine content may be due to the fact that the skin of the fish was discarded before grinding it, and the fish were relatively smaller than the average size of a mature fish of the same

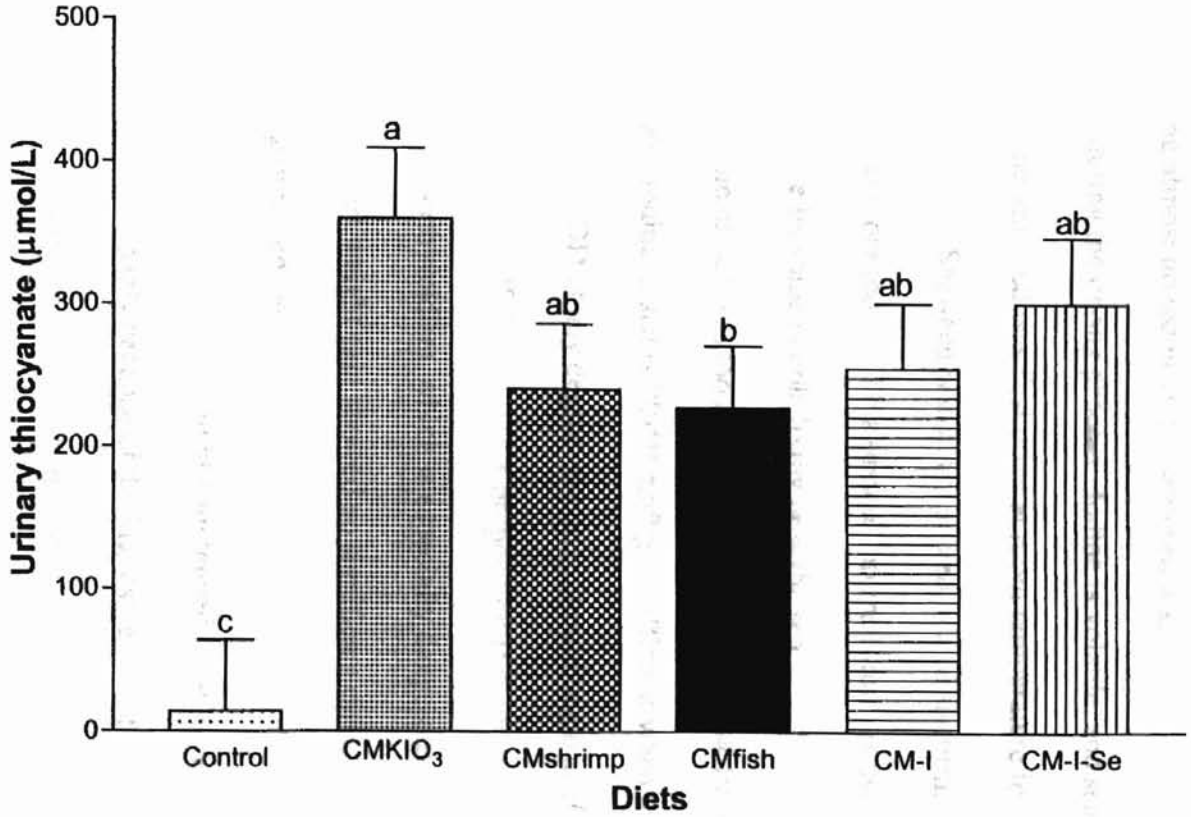

Figure 9: Effect of the diets on urinary thiocyanate

Bars with different letters are significantly different ($P<0.05$)

species. Eckhoff and Maage (1997) found the iodine concentration in the skin of fish to be higher than in the fillet, and the concentration in the fillet seemed to increase with fish size. Therefore finding ways of using a larger size of this fish and eating the skin along with it may increase its value as an iodine source.

Finding almost no iodine in the seaweed was unexpected. This may be due to the loss of iodine during the drying process. Iodine is volatile, and seaweed had a large surface area. The seaweed was first dried at room temperature for 3 day, then in an oven at 100 °C for 3 hours. Seaweed was not analyzed in a fresh form, but eating it in fresh form may provide more iodine. This seaweed in fresh form is usually cooked as sauce and eaten with rice by some people living near the sea.

The sample of salt was too high in iodine (203.24 ± 34.19 mg/kg). According to Stanbury (1996), iodine in salt at 20-50 mg/kg is sufficient when there is no IDD, however, if there is IDD of any degree, the level should begin at 10 mg/kg and not be increased for at least a half decade. This proposition is made to limit the incidence of iodine induced hyperthyroidism, which is especially dangerous for the elderly.

Our results on weight gain showed no significant difference between the control group and the groups fed cassava and millet without added iodine and the groups fed fish and shrimp-supplemented diets. Weight gain tended to be lower in the control group than in any other group, probably because of the highest levels of thyroid hormones that might have made the rats in the control group more active. This was consistent with the findings of Ermans et al. (1980) who found that chronic feeding of SCN or of cassava for two to five weeks did not significantly modify the weights of rats as compared to the control. However, Barrett et al. (1978) found a decreased weight gain and feed

consumption with the ingestion of diets containing 1200 ppm or more of KCN to weanling male rats for 8 weeks. But the ingestion of diets containing 800 ppm or less did not affect weight gain.

To adjust for the effects of body size in the present experiment, the mean thyroid weight was expressed in mg per 100 g body weight of the rats. Thyroid weights for rats on iodine deficient diets, CM-I and CM-I-Se (14. 82 ± 1.54 and 14.87 ± 1.54 mg/100 g respectively), were similar to the ones found by Ermans et al. (1980). In their study, 6 rats fed an iodine-deficient diet with 5 mg SCN per day for 46 days had a mean thyroid weight of 14.7 ± 1.6 mg/100 g. The value for rats fed the control diet in our study was somewhat higher (9.74 ± 1.44 versus 6.4 ± 0.09). One reason for this difference may be that their control diet provided more iodine than our control diet (5 μg iodine per day). Rats fed our control diet had a mean daily iodine intake of 2.17 ± 0.02 μg. However, this amount of iodine as potassium iodate in our control was sufficient, based on American Institute of Nutrition (AIN) recommendations for growing rats (Reeves et al. 1993), and goiter is supposed to be prevented in young rats by supplying 1 to 2 μg per day as iodide (Remington et al. 1936). Another possibility is that the soy protein in our control diet contained some thiocyanate.

The thyroid weight found by Levine et al. (1932) in 193 young rats fed a goiter producing diet (yellow corn 76.0 parts, wheat gluten 20.0 parts, calcium carbonate 3.0 parts, and sodium chloride 1.0 part with 15 μg iodine per kg diet) for 5 weeks led to a mean thyroid weight of 53.2 ± 0.92 mg/100g body weight. This was far greater than the value observed in rats on our iodine deficient diets with cassava and millet, but Levin's diet may have been even lower in iodine than our iodine-depleted diets. When

Remington and Levin (1935) did five successive assays in the same conditions with 8 to 9 rats per group for 5 weeks, the mean thyroid weight varied from 23.9 ± 0.9 to 39.4 ± 1.7 mg/100g body weight. These values were still higher than ours, probably because our iodine deficient diets were adequate in most other nutrients required for normal thyroid function. This confirms the idea that iodine bioavailability is dependent on the general nutritional status of the individual.

The mean thyroid weight in our control group (9.74 ± 1.14 mg/100 g body weight) was similar to that found by Ruz et al. (1999) in 19 weanling rats (21 days old) fed diets that met AIN guidelines for 6 six weeks (9.3 ± 2.1 mg/100 g body weight). However their iodine depleted rats had higher thyroid weight than ours (23.7 ± 4.7 versus 14.82 ± 1.54 for iodine depleted rats, and 23.5 ± 5.8 versus 14.87 ± 1.54 for iodine and selenium depleted rats). This might have been due to the one week longer experiment for Ruz et al. Furthermore, their iodine-depleted diet may have been lower than ours, or the iodine status of the breeding colony from which the weanling rats were obtained may have been different.

The mean serum thyroxine was greater in the control than in any other group (47.60 ± 2.19 nmol/L). This value was lower than the median for 15 young rats fed an amino acid-based diet that met AIN guidelines for six weeks found by (86.2 nmol/L) (Ruz et al. 1999). We do not have enough information about the radioimmunoassay method used by Ruz and colleages, but the thyroxine values for our iodine-depleted rats were quite similar to the values, which they reported.

Urinary thiocyanate was lower in the groups fed fish and shrimp-supplemented diets as compared with the group fed adequate iodine from KIO_3. This may be explained

by the fact that soy protein, which may be a source of thiocyanate, was partially replaced by fish and shrimp in the CMfish and CMshrimp diets respectively. The significantly higher serum and urinary thiocyanate in all the groups of cassava and millet containing diets in comparison to the control confirmed the existence of cyanide or cyanide generating substances in the sample of cassava we used. The presence of thiocyanate in serum and urine of the control group does suggest that either soy or cornstarch are sources of a small amount of thiocyanate.

Urinary iodine is a good indicator of recent iodine intake (ICCIDD 1999), but it seems to be increased by thiocyanate ingestion (Ermans 1981). The mean urinary iodine tended to be higher in CMKIO$_3$ and CMshrimp than in the control, but the difference was not significant. According to Ermans (1981), thiocyanate interferes with the uptake of iodine by the thyroid gland and therefore increases iodine excretion in the urine (Ermans 1981). Cassava, ingested in considerable amounts by the inhabitants of Idjwi (Kivu), Zaire, inhibited the uptake of ^{131}I by 50% in comparison to the control and doubled the excretion of stable iodine in the urine (Ermans 1981). In addition, an acceleration of the exit rate of thyroidal iodine (as determined by the amount of radioiodine retained by the thyroid gland 10 days after ingestion) was observed in rats fed with low concentrations of thiocyanate in comparison with the control (Ermans 1981)

We were not able to confirm that selenium deficiency may worsen iodine deficiency, because there was not a significant difference between the diets CM-I and CM-I-Se diets in any of the indicators of iodine status. This may be due to the fact that some of the dietary components were not completely deficient in selenium. One of the limitations of this study was that we did not determine the selenium content of the food

samples. The principle objective of this thesis was to investigate interactions between cassava and millet containing diets and iodine deficiency. The selenium group was added to the study to see if omitting selenium from the mineral mix would produce enough of a deficiency to affect measured parameters. We assumed that cassava and millet might be low in selenium based on the fact that iodine deficient areas of the world are usually selenium deficient (Hetzel 1988, Utiger 1998). In addition, the prevalence of the myxoedematous form of cretinism, which is associated with a concomitant existence of iodine and selenium deficiencies (Delange 2000), in Guinea let us to suspect that the samples might be selenium deficient. However selenium in food is often associated with the protein source and the soy protein may have contributed some selenium. Selenium frequently replaces sulfur in amino acids. Therefore, our selenium-depleted diet might have been only marginally deficient in selenium. Legumes such as soy are relatively good sources of methionine. Ruz et al. (1999) also did not find any significant difference in indicators of iodine status (weight gain, thyroid weight, thyroxine and T_3) between rats fed with iodine-depleted diets and those fed with diets depleted of both iodine and selenium. However, his low selenium diet was identified only as less than 50 μg selenium /kg diet and investigations using Torula yeast-based diets to produce selenium deficiency have less than 20 μg Se/kg diet. Hotz et al. (1997) also found that combined iodine and selenium depletion in rats did not further antagonize thyroid hormone metabolism beyond what was observed with iodine deficiency alone in male weanling Sprague Dawley rats fed AIN G 93 diets with modified selenium and iodine concentrations. But the diet of Hotz et al. also was not completely selenium-deficient. By analysis it contained 0.05 μg Se/kg diet, which may not be low enough to produce

selenium deficiency in young rats. Additional studies are needed to determine if a severe selenium deficiency combined with iodine deficiency may further impair iodine status.

CHAPTER V

CONCLUSIONS, IMPLICATIONS, AND SUGGESTIONS FOR RESEARCH AND POLICY

The major conclusions that may derived from this study are:

1. A diet composed of cassava and millet decreased iodine bioavailability to rats compared with a diet of cornstarch. This was shown by a significant decrease of serum thyroxine in the rats fed with cassava and millet with adequate iodine from potassium iodate (KIO_3) in comparison with rats fed with the control diet. Significantly higher serum and urinary thiocyanate in all the rats fed with cassava and millet, compared with the control group consuming a cornstarch-based diet, explained the decreased bioavailability.
2. The incorporation of 6.5% of shrimp (adequate iodine) in the cassava and millet diet instead of KIO_3 improved iodine status as much as KIO_3 did in rats. This was shown by the lack of difference in serum thyroxine levels, thyroid weight, and urinary iodine between the group fed cassava and millet with adequate iodine from KIO_3 and the group fed cassava and millet supplemented with 6.5% shrimp.
3. The incorporation of 6.5% of fish with low iodine in the cassava and millet diet improved iodine status as compared with the diets without added iodine and was as effective as the use of adequate iodine from KIO_3 or shrimp for the parameters we measured. This was shown by significantly higher serum thyroxine levels, significantly lower thyroid weight, and significantly higher urinary iodine in the

group fed the fish-supplemented diet as compared with the groups fed iodine-depleted diets. It was also shown by the lack of significant differences in thyroxine, thyroid weight, and urinary iodine between the groups fed adequate iodine from KIO_3 or shrimp and the group fed fish-supplemented diet.

4. The degree of selenium depletion that was achieved with the cassava, millet, and soy protein diet did not worsen iodine deficiency in rats. This was shown by the lack of difference between the group on the iodine-depleted diet and the group on both iodine and selenium-depleted diet in the indicators of iodine status we determined.

This study has very important implications in that it promotes a key factor for self-sufficiency in terms of food and nutrition, which is the use of the local food supply to help with nutritional problems. The study is in concordance with the strategy number 7 (fight against micronutrient deficiency) and its project number 13 (fight against iodine deficiency in Guinea) of the Guinean National Nutrition Policy (Guinean Ministry of Health 1994). The study is also in concordance with the Food and Agriculture Organization of the United Nations (FAO) project: Increasing the use of fish in the alleviation of undernutrition (Eckhoff and Maage 1997).

Based on the findings from this project, we suggest that the following be carried out in Guinea, West Africa.

1- Performance of a sensory study of fish or shrimp-supplemented ''tô'' to see if the supplementation will be accepted by people who usually eat the non-supplemented ''tô''.

2- Determination of iodine bioavailability in fish and shrimp-supplemented ''tô'', by feeding some people with the cooked dish to see if the beneficial effects observed with

fish and shrimp in rats are maintained in humans subjects.

3- Evaluation of the iodine levels obtained by the salt iodization plant in Guinea provided by UNICEF, in order to serve more people and prevent iodine toxicity.

4- Promotion of nutrition education relative to the use of sea products by the population to solve some nutritional problems.

5- Evaluation of the cost and possibilities of production and transport of seafoods to the endemic areas.

6- Investigation of other underused sea products for the possibility of using them as sources of iodine, protein, iron, calcium, vitamin A, selenium or other essential nutrients.

7- Additional laboratory research with very low selenium diets in combination with iodine deficiency to evaluate effects on parameters related to iodine status.

8- Analysis of seafood products from Guinea for the possible presence of toxic trace minerals and organic compounds.

Some of the suggestions listed above may also be applied to other developing countries where populations have iodine deficiency.

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APPENDICES

APPENDIX A

Preparation of The Reagents for the Determination of Iodine in Foods and Urine Samples

Chloric Acid

Weigh 500 g potassium chlorate ($KClO_3$-Fisher, Cat. No-P253-100) and add 910 ml of deionized water. Heat for four hours (under the hood). Let cool and slowly add 375 ml 70% perchloric acid ($HClO_4$- Fisher, Cat. NoA-229 8l b) while stirring constantly. Store the solution in a refrigerator (4 °C) overnight. Filter and store in a dark bottle at 4°C.

2 N Sulfuric Acid (H_2SO_4)

Slowly add 56.2 mL of concentrated sulfuric acid into a 1L volumetric flask containing a small amount of deionized water. Dilute to mark with deionized water.

Arsenious Acid

In a 2 liter volumetric flask, dissolve 20 g arsenic trioxide (As_2O_3-Fisher, Cat. No A59I-100), 50 g sodium chloride (NaCl) in 1 liter 2 N sulfuric acid with heating for at least four hours (under the hood). Cool and dilute to 2 liters with deionized water. Filter and store in a dark bottle at room temperature.

3.5 N Sulfuric Acid (H_2SO_4)

Slowly add 98.3 mL of concentrated acid into a 1L volumetric flask containing a small amount of deionized water. Dilute to mark with distilled water.

Ceric Ammonium Sulfate Solution

Dissolve 48 g ceric ammonium sulfate (Fisher, Cat. No C249 500) in 1L 3.5N sulfuric acid. Store in a dark bottle at room temperature.

Standard Potassium Iodate (KIO_3, Cat. No P253 100)

-Stock solution (1 mg/mL): In a 25 mL volumetric flask, dissolve 0.025g of potassium iodate. Dilute to mark with deionized water. Store in a dark bottle.

-Working standard solution (1 ug/mL): In a 100 mL volumetric flask, pipet 100 µL of the stock potassium iodate solution. Dilute to mark with deionized water. Store in a dark bottle

Test Procedure

Prepare the following standards by pipetting in duplicate the corresponding volumes into a 13 x100 mm test tube

Standard Concentrations (µg iodine/dl)	Volume of working standard (µL)	Volume of water (µL)
0	0	250
2	5	245
5	12.5	237.5
10	25	225
15	37.5	212.5

Pipette 250 µL of sample in duplicate. Add 750 µL of chloric acid solution. Vortex and mark the volume using a marking pen. Heat the tubes for 1 hour in a block set at 110 - 115°C. (under the hood with a trap). Cool the tube and make it to 1 mL mark with deionized water if there is some evaporation. Add 3.5 mL arsenious acid solution to each tube. Vortex and let stand for 15 minutes at room temperature. At one minute time intervals add 350 µL of ceric ammonium sulfate to each tube. At exactly 20 minutes after adding ceric ammonium sulfate, read the absorbency of each tube at 405 nm. Use a cuvette with deionized water as blank.

APPENDIX B

Composition of the Vitamin Mix

Vitamin	g/kg mix
Nicotinic acid	3.000
Ca pentothenate	1.600
Pyridoxine-HCl	0.700
Thiamin-HCl	0.600
Riboflavin	0.600
Folic acid	0.200
D-Biotin	0.020
Vitamin B12 (cyanocobalamin in 0.1% manitol)	2.500
Vitamin E (all-rac-α-tocopheryl acetate) (500 IU/g)	15.00
Vitamin A (all trans Retinyl palmitate)(500,000 IU/g)	0.800
Vitamin D3 (cholecalciferol) (400,000 IU/g)	0.250
Vitamin K (phyloquinone)	0.075
Powdered sucrose	974.655

APPENDIX C

Composition of the mineral mix

Mineral	g/kg mix
Essential mineral elements	
Calcium carbonate, anhydrous, 40.04% Ca	357.00
Potassium phosphate, monobasic, 22.76% P; 28.73% K	250.00
Sodium chloride, 39.34% Na; 60.66% Cl	74.00
Potassium sulfate, 44.87% K; 18.39% S	46.60
Potassium citrate, tri-potassium, monohydrate, 36.16% K	28.00
Magnesium oxide, 60.32% Mg	24.00
Ferric citrate, 16.5% Fe	6.06
Zinc carbonate, 52.14% Zn	1.65
Manganous carbonate, 47.79% Mn	0.63
Cupric carbonate, 57.47% Cu	0.30
*Potassium iodate, 59.3% I	0.01
**Sodium selenate, anhydrous, 41.79% Se	0.01025
Ammonium paramolybdate, 4 hydrate, 54.34% Mo	0.00795
Potentially beneficial mineral elements	
Sodium meta-silicate, 9 hydrate, 9.88% Si	1.45
Chromium potassium sulfate, 12 hydrate, 10.42% Cr	0.275
Boric acid, 17.5% B	0.0815
Sodium fluoride, 45.24% F	0.0635
Nickel carbonate, 45% Ni	0.0318
Lithium chloride 16.38% Li	0.0174
Ammonium vanadate, 43.55% V	0.0066
Powdered sucrose	209.806

*The mineral mix for the CM-I diet (cassava and millet without added iodine) was prepared without adding potassium iodate.

**The mineral mix for the CM-I-Se diet (cassava and millet without added iodine and selenium) was prepared without adding either potassium iodate and sodium selenate.

APPENDIX D

Oklahoma Animal Disease Diagnostic Laboratory

Accession: 00040665

Oklahoma State University

Report date 4-21 –OO/GAC
Owner: Arjmandi/Stoker Res
Coordinator: Greg A. Campbell, DVM PhD
CI @@

P,O. Box 7001
Stillwater, OK 74076-7001
(405) 744-6623
FAX: 744-8612

ARJMANDI/STOKER RESEARCH (Acct: 4672)
ATTN: LANGE, DOUGLAS
NUTRI SCI DEPT/416 HES
ACCT# AE-5-55560
STILLWATER, OK 74078

SUBMISSION SUMMARY

Taken: not given
Received: 4-1 0-00

Species	Animals	Tests	Completed
Rodent	I	0	0

**** * FINAL REPOR T * ****

DIAGNOSIS AND COMMENTS 4-21-00/4:11p

DIAGNOSES	DATE
Thyroid, within normal limits.	4-21-00

Comments:

Gross description:
Six tubes containing small tan 1-2 mm soft tissue specimens are received. They are labeled 'Thyroid #4,' 'Thyroid #14," 'Thyroid #36," 'Thyroid #39," "Thyroid #42,' 'Thyroid #48.' These are submitted in cassettes 1-6, respectively.

Microscopic description:
Multiple sections are examined on 6 slides. All consist of thyroid within which there are no histologic lesions. A few contain small sections of parathyroid gland.

Comment:
Thyroid gland was present in all sections examined.

APPENDIX E

OKLAHOMA STATE UNIVERSITY

OSU

Institutional Animal Care and Use Committee
Stillwater, Oklahoma 74078
405-744-7631

February 4, 2000

Institutional Animal Care and Use Committee Action

This protocol was reviewed by the IACUC with the following action:

Principle Investigator: Dr. Barbara Stoecker
Department: Department of Nutritional Sciences

Protocol Title: Effects of Fish on Bioavailability of Iodine from Cassava

Protocol Number: 827

Animal Number and Species: 40 rats

Expiration Date: 1/31/03

Approval ____XX____ Deferral _____

Approval with Modifications _____

Comments:

Date of final Institutional Committee Action 2/1/00

Signature of IACUC Chairman _____ Date 2/8/00

Signature of IACUC Veterinarian K Vargo Date 2/9/00

Institutional Assurance Number A3722-01

For Committee Administrative Purposes Only

____ Additional information was requested, and has been provided by P.I.

____ Significant modifications to ACUF were requested (see attached information).

APPENDIX F

Components of the Kit for Thyroxine Analysis

Total T_4 antibody coated tubes (TT41)
The kit contained 100 polypropylene tubes (colored in light green) coated with antibody to T_4 and packaged in zip-lock bags. Tubes were to be Stored refrigerated and protected from moisture and carefully resealed in bags after opening. The kit was said to be stable at 2-8°C until the expiration date marked on the bag.

^{125}I Total T_4(TT42)

One vial (colored in red) of iodinated T_4, ready to use, with blocking agents to thyroid hormone-binding proteins. The vial contained 105 ml. It was said to be stable at 2-8°C for 30 days after opening or until the expiration date indicated on the vial.

Total T_4 Calibrators (T4C3-8)

One set of six vials, labeled A through F, of T_4 calibrator in processed human serum. The calibrators were supplied in liquid form, ready to use. The zero calibrator vial A contained 2.0 ml and each of the remaining calibrator vials B through F contained 1.0 ml. The calibrators were said to be stable at 2-8°C after opening. For longer storage it was recommended to aliquot and freeze them at -20°C for up to 6 months. The calibrators contained, respectively, 0, 1, 4, 10, 16, and 24 µg of T_4 per deciliter processed human serum.

Test Procedure

All components were at room temperature (15-28°C) before use.
Four plain (uncoated) 12 x75 mm polyethylene tubes T (for total count) and NSB (nonspecific binding) were labeled in duplicate. Then twelve Total T_4 antibody-coated Tubes A (maximum binding) and B through F were labeled in duplicate. After that, additional antibody coated tubes were labeled in duplicate for the samples. The concentrations of the calibrators A through F were 0, 1, 4, 10, 16, and 24 µg/dl respectively. For the NSB and A tubes 25 µl of the zero calibrator A was pipetted, and 25 µl of each remaining calibrator, control and samples were pipetted into the remaining prepared tubes. Then 1.0 ml of ^{125}I total T_4 was added to every tube, and the tubes were vortexed. After that, the tubes were incubated for 60 minutes at 37°C in a water bath. Thereafter, the tubes were thoroughly decanted (except the total count tube), removing all visible moisture by striking the tubes sharply on a absorbent paper. After that, the tubes were placed in a gamma counter for 1 minute per tube. Then sample values were derived from the standard curve.

APPENDIX G

Preparation of the Reagents for Serum and Urinary Thiocyanate Analysis

For about 200 samples, the following reagents were prepared: we prepared the following:

1-Hydrchloric acid (HCl) 1 M

82.6 ml of concentrated HCl (12.1 mol/L) were diluted to 1 L with deionized water.

2- Potassium thiocyanate standard solutions (KSCN)

A stock solution of 100.0 mmol/L was first prepared by diluting 0.9718g of KSCN from Sigma Chemical Company (catalogue No P3048) to 100 mL with deionized water. Then the working standards of concentrations 20, 50, 100, 200, and 300 μmol/L were prepared from the stock solution by diluting respectively 20 μL, 50 μL, 100 μL, 200 μL and 300 μL of the stock solution to 100 mL with deionized water.

3- Sodium hydroxyde (NaOH) 1 M

40 g of NaOH bought from E.M. Science (catalogue no SX0590-3) were diluted to 1 L with deionized water.

4- Sodium perchlorate ($NaClO_4$) 1 M

140.5 g of $NaClO_4$ bought from Sigma Chemical Company (catalogue no S3546), were diluted to 1 L with deionized water.

5- Acetic acid 0.35 M

2.00 mL glacial acetic acid (17.4 M) were diluted to 100 mL with deionized water.

6- Sodium hypochlorite 50 mmol/L

2 mL of a stock solution of sodium hypochlorite (0.5 mol/L disolved in NaOH 0.1 mol/L) bought from BDH Chemicals (reagent no 230394M) were mixed with 18 mL deionized water to make 20 mL of the solution with 50 mmol/L

7- Color reagent

3.7 g of NaOH were disolved in 200 mL deionized water, and then 7 g of 1,3 dimethyl barbituric acid and 5.72 g isonicotinic acid were added.

Other Material Needed

- 50 Poly-prep chromatography columns (0.8 x 4 cm) purchased from Bio-Rad Laboratories (catalogue no 731-1550)

-Weakly basic, Amberlyst A-21 ion-exchange resin purchased from Rohm & Haas Co (catalogue no 21,641-O)

Procedure for Serum and Urinary Thiocyanate Analysis

The resin was dried in an oven for 24 hours at 100°C. Then it was slurried into two volumes of deionized water and allowed to sediment for 5 - 10 minutes. The supernatant containing fine particles was decanted to ensure an optimal column flow rate and the procedure was repeated 3 times. The resin was transferred to the chromatographic columns to make about 1-ml column of the resin (2.5 x 0.7 cm column). Then the resin was washed with 3 volumes of HCl 1mol/L, followed by deionized water to neutral pH (pH indicator paper). Then the resin was washed with 10 volumes of NaOH 1 mol/L, followed by deionized water to neutral pH. The resin was supposed to be stable when stored at 4°C for at least one year.

We diluted 0.5 ml aliquot of blank, working standards, or urine samples with 5 ml of NaOH 1 mol/L, and applied to the 2.5 x 0.7 cm column of Amberlyst A-21. The column was washed three times with 5 mL portions of water , and thiocyanate was eluted by 8 ml of $NaClO_4$ 1 mol/L. To a 2-mL of the eluate, we added 0.1 mL of acetic acid 0.35 mol/L, and mixed on a Vortex mixer. The chlorinating reaction was then performed by adding 0.05 mL of sodium hypochlorite 50 mmol/L, and the sample was again mixed. Then within 2 minutes, 0.3 ml of the color reagent was added. After 10 minutes, the absorbance was read at 607 nm and the amount of thiocyanate was calculated from a calibration graph.

Daily Iodine Intake and Effect of the Diets on Weight Gain and Organ Weight of the Rats

	Control CornsKIO$_3$	CMKIO$_3$	CMshrimp	CMfish	CM-I	CM-I-Se
Daily iodine intake (µg)	2.17 ± 0.02^{a}	2.21 ± 0.02^{a}	2.22 ± 0.02^{a}	0.32 ± 0.02^{b}	0.00 ± 0.02^{c}	0.00 ± 0.02^{c}
Weight gain (g)	113 ± 5^{c}	122 ± 5^{bc}	128 ± 5^{ab}	138 ± 4^{a}	118 ± 5^{bc}	118 ± 5^{bc}
Thyroid weight (mg)	14.67 ± 2.35^{b}	20.21 ± 2.35^{b}	17.36 ± 2.35^{b}	15.42 ± 2.21^{b}	23.75 ± 2.35^{a}	24.80 ± 2.51^{a}
	$(9.74 \pm 1.44)^{b}$	$(11.95 \pm 1.44)^{ab}$	$(10.03 \pm 1.44)^{b}$	$(8.60 \pm 1.36)^{b}$	$(14.82 \pm 1.54)^{a}$	$(14.87 \pm 1.54)^{a}$
Liver weight (g)	5.58 ± 2.28^{a}	5.76 ± 0.28^{a}	6.05 ± 0.27^{a}	6.91 ± 0.27^{b}	5.95 ± 0.28^{a}	6.01 ± 0.28^{a}
	$(3.60 \pm 0.14)^{a}$	$(3.40 \pm 0.14)^{a}$	$(3.50 \pm 0.14)^{a}$	$(3.82 \pm 0.14)^{a}$	$(3.74 \pm 0.15)^{a}$	$(3.66 \pm 0.14)^{a}$
Kidney weight (g)	1.37 ± 0.04^{a}	1.29 ± 0.04^{a}	1.44 ± 0.03^{a}	1.36 ± 0.03^{a}	1.23 ± 0.04^{b}	1.24 ± 0.04^{b}
	$(0.88 \pm 0.02)^{a}$	$(0.76 \pm 0.024)^{b}$	$(0.84 \pm 0.024)^{a}$	$(0.75 \pm 0.022)^{b}$	$(0.77 \pm 0.025)^{b}$	$(0.76 \pm 0.024)^{b}$
Spleen weight (g)	0.46 ± 0.03^{b}	0.45 ± 0.03^{b}	0.46 ± 0.03^{ab}	0.55 ± 0.03^{a}	0.45 ± 0.03^{b}	0.45 ± 0.03^{b}
	$(0.29 \pm 0.014)^{a}$	$(0.27 \pm 0.014)^{a}$	$(0.27 \pm 0.014)^{a}$	$(0.30 \pm 0.013)^{a}$	$(0.28 \pm 0.015)^{a}$	$(0.28 \pm 0.014)^{a}$

Values are means ± SE of the means; n = 8 or 9. Values with different superscripts for the same parameter are significantly different ($P<0.05$). Values in parentheses are weights in mg/100g body weight for thyroid and percents of body weights for the other organs.

Corns = cornstarch. KIO_3 = potassium iodate. CM = cassava and millet. fish = low iodine from fish. shrimp = adequate iodine from shrimp. -I = without added iodine. -I-Se = without added iodine and selenium.

Effect of the Diets on Serum Thyroxine, Serum Thiocyanate, Urinary Iodine, and Urinary Thiocyanate of the Rats

Parameters	Control CornsKIO$_3$	CMKIO$_3$	CMshrimp	CMfish	CM-I	CM-I-Se
Serum thyroxine						
(nmol/L)	47.60 ± 2.19^a	35.73 ± 2.19^b	36.51 ± 2.19^b	31.86 ± 2.19^b	20.77 ± 2.19^c	19.35 ± 2.19^c
(μg/dL)	(3.69 ± 0.17)	(2.77 ± 0.17)	(2.83 ± 0.17)	(2.47 ± 0.17)	(1.61 ± 0.17)	(1.50 ± 0.17)
Serum thiocyanate						
(μmol/L)	46.06 ± 12.15^c	146.69 ± 12.15^a	159.11 ± 11.46^a	142.27 ± 11.46^a	107.22 ± 12.99^b	132.83 ± 12.15^{ab}
(mg/dL)	(0.27 ± 0.07)	(0.85 ± 0.07)	(0.92 ± 0.06)	(0.83 ± 0.06)	(0.62 ± 0.08)	(0.77 ± 0.07)
Urinary Iodine (μmol/L)	0.80 ± 0.09^a	0.90 ± 0.09^a	0.89 ± 0.09^a	0.69 ± 0.08^a	0.15 ± 0.09^b	0.20 ± 0.09^b
(μg/dL)	(10.14 ± 1.14)	(11.42 ± 1.14)	(11.24 ± 1.14)	(8.73 ± 1.07)	(1.85 ± 1.14)	(2.64 ± 1.14)
Urinary thiocyanates						
(μmol/L)	14.41 ± 49.28^c	60.09 ± 49.28^a	240.14 ± 46.09^{ab}	227.22 ± 43.46^b	254.79 ± 46.09^{ab}	300.80 ± 46.09^{ab}
(mg/dL)	(0.08 ± 0.28)	(2.09 ± 0.28)	(1.39 ± 0.26)	(1.32 ± 0.25)	(1.48 ± 0.26)	(1.74 ± 0.26)

Values are means ± SE of the means; n = 8 or 9. Values with different superscripts for the same parameter are significantly different (P <0.05).

Corns = cornstarch. KIO_3 = potassium iodate. CM = cassava and millet. fish = low iodine from fish. shrimp = adequate iodine from shrimp. -I = without added iodine. -I-Se = without added iodine and selenium.

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Master of Science

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