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Effects of Roasted Beef Based-diet on Thyroid Hormones and Vitamins: the Biochemical Roles of *Lycopersicon esculentum* L. and *Allium cepa*

[1]*Ujowundu, C. O., [1]Nwaoguikpe, R. N., [2]Belonwu, D. C., [1]Ujowundu F. N. and [1]Igwe K. O.

[1]Department of Biochemistry, Federal University Technology Owerri, Nigeria.
[2]Department of Biochemistry, University of Portharcourt, Nigeria.

ABSTRACT: In this 21 days study, two biomarkers- thyroid hormones and vitamins were used to evaluate the hormonal disruptive potentials of locally processed beef and the protective effects of ethyl acetate and methanol extracts of *Lycopersicon esculentum* L. and *Allium cepa* respectively. Twenty five male rats (168.8±13.05 g) were divided into 5 groups of 5 rats each. Feed was formulated with 15% w/w of processed beef. Group I (control) rats were fed rat pellets (RP) only, group II received formulated diet (FD) only, group III and IV received FD and 500 mg/kg bw of *A. cepa* and *L. esculentum* L. extracts respectively and group V received FD 250 mg/kg bw each of *A. cepa* and *L. esculentum* L extracts. Serum concentrations of thyroxine (T_4), triiodothyronine (T_3), and thyroid stimulating hormone (TSH) were determined by an ELISA method used for quantitative *in-vitro* determinations. Vitamins were determined spectrophotometrically. Significant ($P < 0.05$) disturbances in serum T_3, T_4 and TSH concentration were observed in rats exposed to formulated diet only when compared to the control. However, none significant ($P < 0.05$) changes in thyroid hormones concentration were observed in groups administered the extracts, compared to the control. There were also, significant ($P < 0.05$) decrease in concentrations of vitamins A, C, D and E in FD group when compared to RP groups or groups administered either of the extracts or the combination. This study indicates that, these disturbances which could result to adverse biochemical and physiological consequences could be ameliorated if consumers of roasted beef avail themselves of the chemoprotective potentials of *L. esculentum* L. and *A. cepa* sauce.

KEYWORDS: Polycyclic aromatic amine, Heterocyclic aromatic amines, Roasted beef, Toxicants, Antioxidants, Lycopene, Flavonoids

1.0 Introduction

Thyroid gland and thyroid hormone disrupting chemicals are xenobiotics that alter the structure and/or function of the thyroid gland. They can alter regulatory enzymes associated with homeostasis of thyroid hormone, or change the circulating or tissue concentrations of the hormone. Thyroid hormones function in concert with other hormones and/or vitamins to affect metabolic and physiological processes. A complex interplay involving the hypothalamus, pituitary and thyroid gland is required for proper regulation of thyroid function (Lechan and Fekete, 2006). Normal thyroid functions ensure functional vertebrate system homeostasis, which is important in metabolism, growth and development (Tata, 1999; Yen 2001). This balance is essential in feotal and neonatal brain development (Porterfield and Hendrich, 1993; Chan and Kilby, 2000).

The growing studies in thyroid function and metabolism have indicated numerous environmental toxicants which influence thyroid functions (Brucker-Davis, 1998; Ujowundu *et al.*, 2013). These environmental chemicals cause their disrupting effect by altering thyroid hormone synthesis, transport and metabolism. Various *in vivo* and *in vitro* studies report that thyroid and vitamin A homeostasis and hormones controlling calcium homeostasis are

Corresponding Author
Tel.: +234 8036683491;
E-mail: ujowundu@yahoo.com

targets of endocrine disrupting contaminants (Lind *et al*., 2004; Boas *et al*., 2006; Novák *et al.*, 2008). A good understanding of how environmental exposures affect the thyroid gland and metabolism is important, especially when the thyroid's self-correcting feedback system fails to re-establish balance. The biotransformation capacity in given specie depends on the compound-specific characteristics, such as molecular structure, physicochemical properties, and on the contaminant exposure (Letcher *et al.*, 2000).

Polycyclic aromatic hydrocarbons (PAH) and Heterocyclic aromatic amines (HAAs) are formed during prolonged cooking (roasting/barbecuing, grilling or pan frying) of meat at high temperature (Felton *et al.*, 1986; Gross and Gruter, 1992). Long term feeding studies using PAHs and HAAs have shown their carcinogenic effects in rats and mice (Ohgaki, 1991). To initiate carcinogenesis, PAHs and HAAs are enzymatically activated to electrophiles to enable their binding to DNA (Hein, 1988). Studies have shown that ripe tomato (*Lycopersicon esculentum L.*) fruits contain a carotenoid pigment known as lycopene, having potential health benefits (Shi *et al.*, 2002; Rao and Rao, 2004). The beneficial effects of lycopene may be related to the molecular structure which contains extensively conjugated double bonds, conferring it antioxidant status. Among carotenoids, lycopene from tomato fruit is the most potent *in vitro* antioxidant (Gerster, 1997). Results from epidemiological and experimental studies support the view that lycopene may provide protection against cardiovascular diseases, type II diabetes mellitus and certain types of cancer (Upritchard *et al.*, 2000; Giovannucci, 2005; Omoni and Aluko, 2005). White and red coloured onions (*Allium cepa L.*) are commonly consumed vegetables all over the world. The use of these vegetables dates back to the ancient times (Banerjee and Maulik, 2002). Onions are good source of vitamin C, B6, biotin and minerals like chromium, calcium and dietary fibre. In addition, they contain good amounts of folic acid and vitamins B1 and K. Onion is a rich source of quercetin, a potent antioxidant flavonoid and a good source of sulfur and sulfur-containing amino acids (methionine and cystine). These compounds are responsible for the hypolipidemic and hypocholesterolemic effects of onions in both human and animal studies (Rahman and Lowe, 2006; Corzo-Martinez *et al.*, 2007).

Many Nigerians process their food especially beef and fish by roasting and smoking, thereby exposing them to the negative biochemical consequences resulting from metabolites of PAH and HAA biotransformation. 'Suya' is the name given to a locally prepared beef in Nigeria. This is a popular delicacy processed by exposing beef to heat and smoke and sometimes to direct flame from fire woods. Some vendors of roasted beef in Nigerians add tomato and onions as sauce when they sell beef meals prepared as such. Some studies have shown that vitamins and thyroid hormones are sensitive biomarkers of environmental chemical exposure (Peakall, 1992; Fox, 1993). However, very few studies have been carried out on the effect of chemical toxicants generated in roasted beef on thyroid and vitamin metabolism. This study evaluated the adverse biochemical implication of roasted beef (Suya) consumption and the benefits derivable from the practice of adding tomato and onion sauce. This is to assess the protective and ameliorative effects for the greater consuming population of Nigeria.

2.0 Materials and Methods

2.1 Collection and authentication of the fruits

Fresh ripe tomato fruits and onion bulbs were bought from Ekeonuwa market in Owerri Municipality, Imo State, Nigeria. The vegetable samples were identified and authenticated by a taxonomist in the Department of Crop Science, Federal University of Technology, Owerri, Imo State, Nigeria.

2.2. Preparation of ethylacetate and methanol extracts

Fresh fruits of *L. esculentum* L. and *A. cepa* were washed with distilled water, homogenized and oven dried at reduced temperature of 40 ^{0}C to reduce the moisture content. Crude lycopene

and flavonoid were extracted from the tomato fruits and onion bulbs by adopting the methods (Fiamegos *et al*., 2004; Benkeblia, 2005; Constantine, 2007; FAO/JECFA, 2009; Rath *et al*., 2012). About 200 g of the dried *L. esculentum* L was divided into four equal amounts (50 g). Each portion was subjected to exhaustive soxhlet extraction process with 45 ml of ethyl acetate and the filtrate was pooled together. The filtrates were concentrated with rotary evaporator and a gel-like residues were obtained. Similar extraction protocol was used for *A. cepa* extraction with methanol. The yield was 43.80 g (21.9 %, w/v) and 62.34 g (31.17 %, w/v) for *L. esculentum* L. and *A. cepa*, respectively. The extracts were stored in the refrigerator for further use. Toxicity studies with various doses of the extracts, established a safe dose of 500 mg/kg. This safe concentration was administered orally to the rats using an intubator for 21 consecutive days.

2.3 Preparation of Beef Meal

A sizeable portion of fresh beef was purchased from a butcher at a mini-market in Obinze, Owerri West LGA, Nigeria. The beef had fatty and flesh parts and both were processed. The fresh beef sample was exposed initially (6-12 hours) to heat, smoke and flame from firewood and then exposed to mild and consistent smoke and heat from firewood for 4 days (6 hours daily). This covers the period of processing a dry delicacy of "suya". The beef was processed by a food vendor under the supervision of the researchers.

2.4 Animal Grouping

Twenty five (25) adult male Wistar albino rats of mean weight of 168.8±13.05 g were divided into five groups with each group containing five rats. The rats were housed in steel cages and allowed to acclimatize for two weeks. Rat feed was formulated with the processed beef and rat pellets. The formulated feed contained 15% by weight of processed beef and 85% by weight of rat pellets. The feed was properly stored in an air-tight plastic container. The extracts were administered orally with an intubator every morning for 21 days to the indicated groups. Group I rats served as negative control and were fed with rats pellets and water only. Group II (positive control) received formulated diet only. Group III rats received the formulated diet and 500 mg/kg of onion extract. Group IV were served the formulated diet and 500 mg/kg of tomato extract. Group V rats were served the formulated diet and 500 mg/kg of combined onion (250 mg/kg) and tomato (250 mg/kg) extracts. The extracts were administered orally. The rats were maintained in a well ventilated room with a temperature range of 22 – 26^{o}C under 12 hours' day and 12 hours night photoperiodicity. This animal study was approved by the Department of Biochemistry Ethics Committee and we adhered to the guidelines outlined for the care and well being of research animals (NIH, 1985).

2.5 Collection of Blood Sample

The animals were sacrificed on the 22nd day of the study after 24 hours fast. Blood was collected from each animal by cardiac puncture with 10 ml sterile needles and syringes. The blood sample obtained from each group was divided into two, one part was allowed to clot and serum was obtained from blood sample for biochemical assay by centrifuging at 2325.44 g for 10 minutes. The other part was poured into sample bottles with anticoagulant.

2.6 Determination of Triiodothyronine, Thyroxine, and Thyroid Stimulating Hormone (TSH)

Triiodothyronine (T_3), thyroxine (T_4) and thyroid stimulating hormone (TSH) were determined using Strepta vidin-biotin kit of TECO diagnostics CA USA. This is an ELISA method for the quantitative *in-vitro* determination based on the principle of solid phase enzyme-linked immunosorbent assay (Utiger, 1974; Engall, 1980).

2.7 Determination of Vitamins A, C, D and E in the Serum

Serum vitamins A and C were determined by the method described by Tietz (1983) which depends on the oxidation of ascorbic acid to

diketogluconic acid in a strong acid solution.Vitamin D was determined according to the method of Pearson (1976).The method described by Association of Vitamin Chemists (1966) was used to determine vitamin E and this was based on the oxidation of the α-tocopherol in alcoholic solution by ferric chloride.

2.8 Statistical Analysis

Results are expressed as mean ± standard deviation of triplicate determinations and data obtained were subjected to analysis of variance (ANOVA) using the statistical package for social sciences (SPSS version 16, Chicago, Illinois). Duncan's multiple range test (Duncan, 1955) was used and statistical significance was considered at $P < 0.05$.

3.0 Results

The values obtained from the determination of T_3 (Figure 1) indicates a significant difference ($P = 0.006$) among the five groups of rats. The mean concentration of T_3 reduced non-significantly ($p= 0.108$) compared to negative control. However, the mean T_3 concentration of rats that were exposed to the formulated diets and received onion, tomato and onion/tomato extracts increased significantly ($p<0.05$), whereas no significant difference ($p= 0.365$) in the mean concentration of T_3 was observed among rats administered the extracts. The rats treated with onion extracts showed the highest mean T_3 concentration. The observed mean concentrations of T_4 (Figure 2) indicates significant difference ($p= 0.005$) among the entire group. Generally, the rats in the group treated with tomato extract only, had the highest mean T_4 concentration and those fed the formulated diet only (untreated rats) had the lowest. The T_4 result show a significant increase ($p<0.05$) in T_4 concentration compared to the rats fed the formulated diet only. Between the positive and negative control, a significant ($p=0.028$) reduction in mean T_4 concentration was observed. There was also a non significant ($p<0.05$) increase in mean T_4 concentration in rats administered the combined onion/tomato extracts when compared with rats fed formulated diet only.

The effect of treatments on mean TSH concentration is presented in Figure 3. TSH concentration varied significantly among the five groups. The highest TSH concentration was observed in rats fed formulated diet only and the lowest in rats administered onion extracts. The mean TSH concentration increased significantly ($p= 0.008$) in rats fed formulated diet only when compared with the rats fed rats' pellets only (negative control). The rat groups which were fed the formulated diet and administered the extracts have significantly reduced ($p<0.05$) TSH concentration when compared with the rats fed formulated diet only. No significant difference ($p= 0.777$) was observed between the groups of rats that received treatment after exposure. The results obtained from the estimation of serum vitamin concentration (Figures 4 and Table 1) show significant reduction ($p<0.05$) in vitamins in rats fed formulated diet only when compared with the control. This indicates adverse metabolic effect on rats exposed to the roasted beef diet. Also, the concentrations of vitamin D and A in rat groups administered the onion and/or tomato extract were maintained within the concentration range obtained in rats fed rats' pellets only. Significant increase was observed in serum concentration of vitamin E in rats administered tomato and onion/tomato extracts when compared with rats fed rat's pellets only (control). Similarly, a significant increase was observed in serum vitamin C in rats administered tomato extracts when compared with the control.

4.0 Discussion

Some studies have indicated that homeostasis of vertebrate endocrine systems can be altered on exposure to environmental xenobiotics (Lilienthal *et al.,* 2000; Boas *et al.,* 2006;

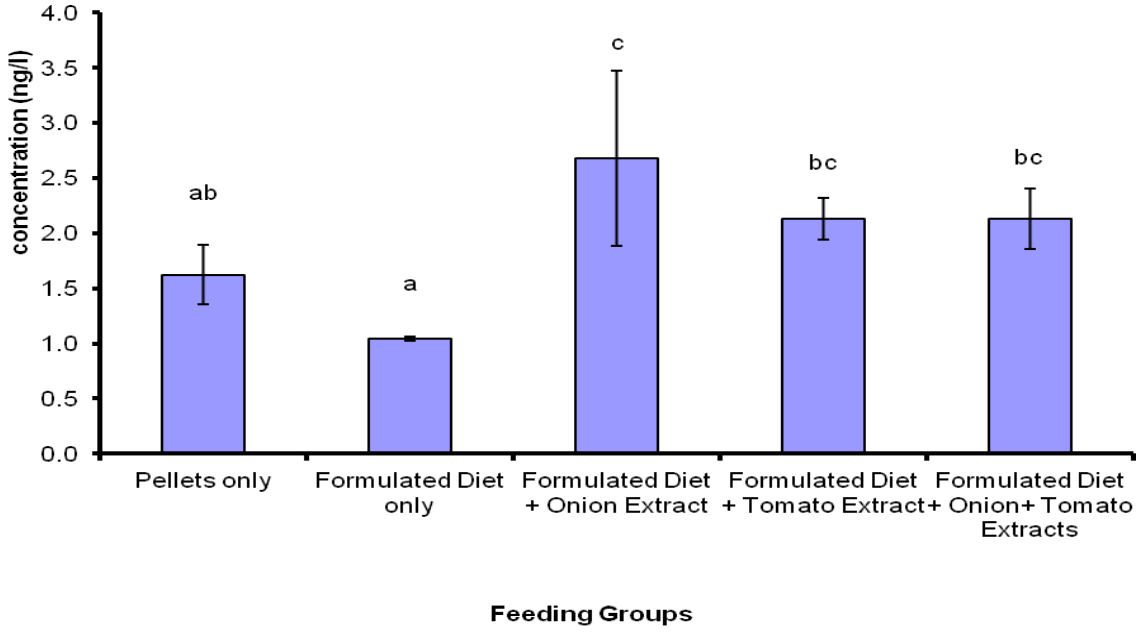

Figure 1: Effects of Exposure to Diets and Extracts of Onion and Tomato onTriiodothyronine Concentration of Albino Rats

Bars with different letters are sigbificantly different (p<0.05)

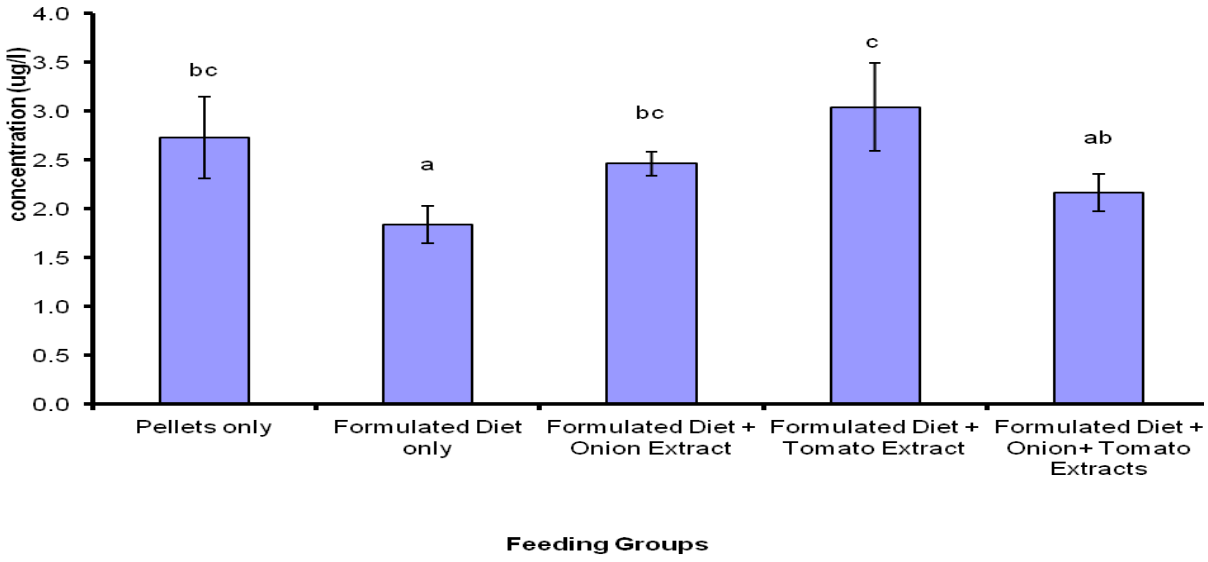

Figure 2: Effects of Exposure to Diets and Extracts of Onion and Tomato on Serum T4 concentration of Albino Rats

Bar with different letters are significantly different (p<0.05)

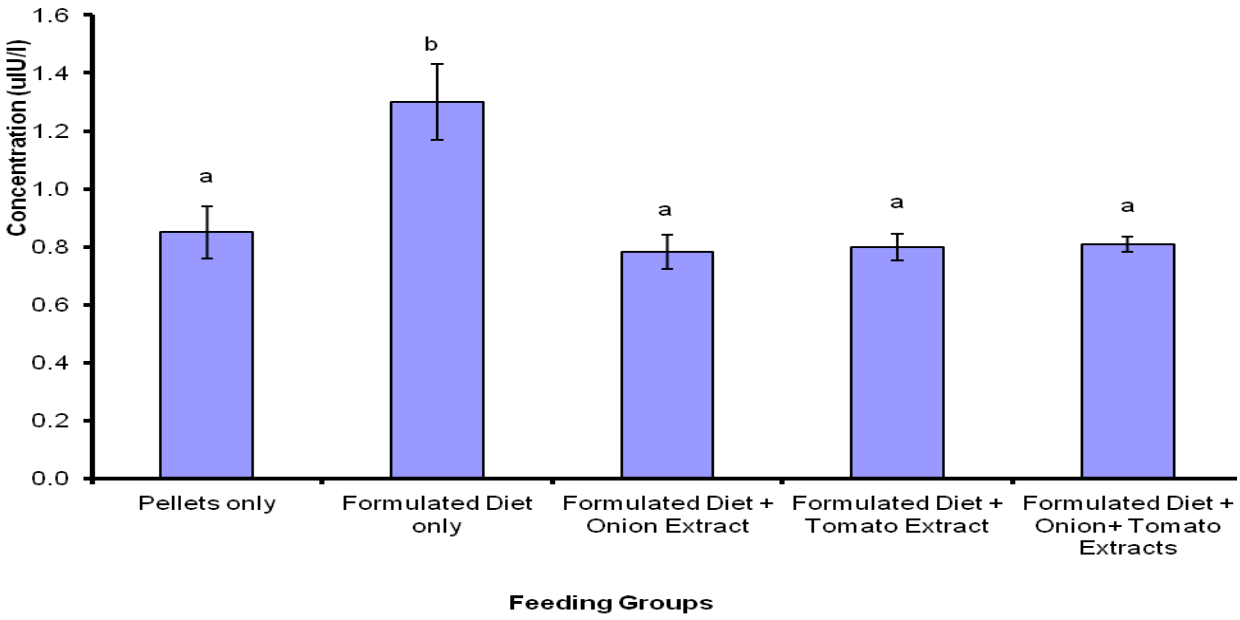

Figure 3: Effects of Exposure to Diets and Extracts of Onion and Tomato on Serum TSH Concentration of Albino Rats

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

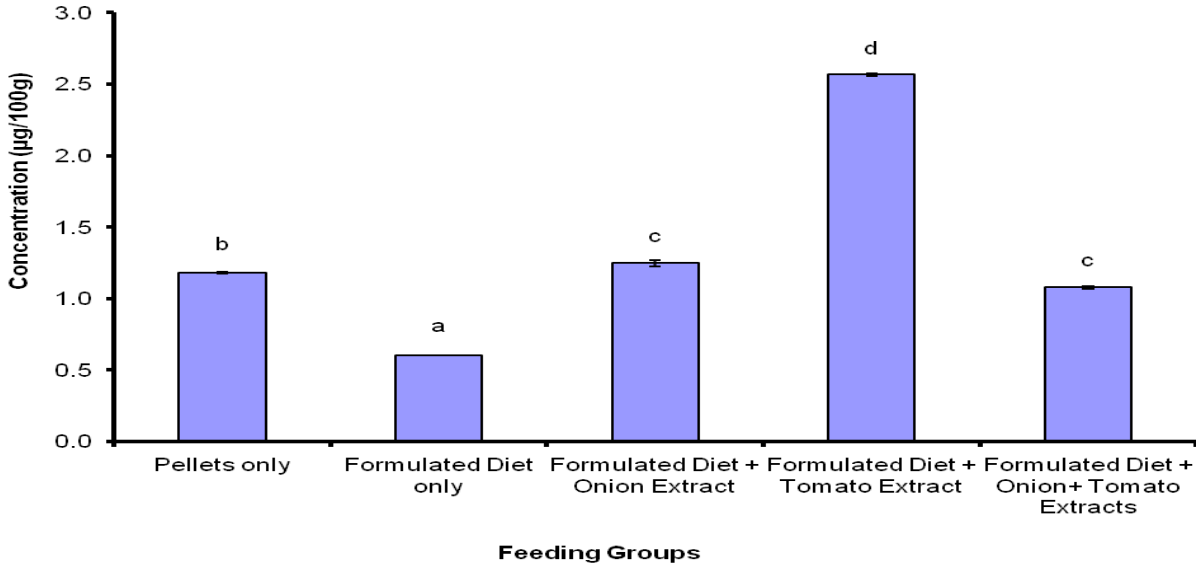

Figure 4: Effect of Exposure to Diets and Extracts of Onion and Tomato on Vitamin A Concentration in Albino Rats

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

Table 1: Effect of exposure to different diets on the concentrations of vitamin D,E and C in albino rats

Groups	Vitamin D	Vitamin E	Vitamin C
Pellets only	0.271±0.002[a]	0.576±0.006[b]	2.694±0.009[a]
Formulated Diet only	0.134±0.003[b]	0.463±0.003[a]	1.208±0.015[b]
Formulated Diet + Onion Extract	0.251±0.004[c]	0.469±0.003[a]	3.022±0.093[c]
Formulated Diet + Tomato Extract	0.325±0.005[d]	0.775±0.006[c]	6.603±0.015[d]
Formulated Diet + Onion+ Tomato Extracts	0.237±0.004[e]	0.729±0.006[d]	2.272±0.039[e]

These values are mean± standard deviation of triplicate determinations. Values in each column with different superscripts are significantly different ($p<0.05$).

Novák *et al.*, 2008). These xenobiotics disrupt thyroid hormone metabolism, altering the structure and function of thyroid gland. Oxidative stress induced by metabolites of PAH and HAA degradation may not only cause signs of hypothyroidism but may also impair the liver's ability to detoxify xenobiotics. Hypothyroidism is the consequence of the suppression of TSH production which results in low thyroid function. The presence of PAHs and other toxic compounds in roasted beef (Ujowundu *et al.*, 2004) used to formulate the diets, fed to the rats may have elicited some metabolic stress.

The influence of xenobiotics in the formulated diet in our feeding study indicates that, T_3 and T_4 concentrations were significantly ($p<0.05$) decreased in rats exposed to formulated diets only. This could indicate inhibition of hormone synthesis and when sustained may degenerate to hypothyroidism or drive the inactivation of T_3 (by converting it to reverse T_3). The observed reduction in T_3 and T_4 concentrations was consistent with previous reports on contaminant-related reductions in thyroid hormone concentrations in animals exposed to contaminants (Brouwer *et al.*, 1989; Chiba *et al.*, 2001; Debier *et al.*, 2005). The reduced thyroid hormones concentration could be linked to the increased FSH in rats fed formulated diet only. TSH signals T_3 and T_4 production and secretion. Similarly, the xenobiotics generated during roasting of the beef (Ujowundu *et al.*, 2014) used in diet formulation may have caused some histopathological lesions in rats' thyroid glands resulting in serum hormone reduction as observed in seals by Schumacher *et al.*, (1993). Also, Brouwer *et al.* (1998) reported that, the disruption in circulatory transportation and altered metabolic enzyme activity as possible mechanisms of altered hormone synthesis in the thyroid gland. However, TSH and T_4 concentrations in rats fed the diet formulated with roasted beef and administered onion and tomato extracts were within the concentration obtained in control rats. This indicates that, the constituents of the extracts (lycopenes and flavonoids) may have improved the physiology of the thyroid gland by preventing cellular and parenchyma damage to the thyroid gland. The position of hydroxyl groups and other features in the chemical structure of flavonoids are important for their antioxidant and free radical scavenging activities. Onions is abundant in a flavonoid called quercetin, a potent antioxidant.

Vitamin A (retinoids) and thyroid hormones play critical roles during development, growth and function throughout life. The values obtained for vitamin A in this study showed that its concentration decreased significantly ($p<0.05$) in rats fed the formulated diet only. This supports the assertion that xenobiotics can disrupt vitamin A metabolism, depleting body stores (Spear *et al.*, 1986; Zile, 1992; Peakall, 1992; Fox, 1993). The observed reduction in serum vitamin A concentration may be associated with the hypothyroid status observed in rats fed formulated diet only, which content may have altered the metabolic rate (Bonet *et al.*

2003). Inadequate vitamin A, limits the ability of thyroid gland to produce TSH, which stimulates T_4 and T_3 synthesis. Also, since vitamin A is required by the body to convert T_4 to T_3, the reduced vitamin A concentration in rats fed formulated diet only, makes it metabolically impossible to adequately utilize T_4 (when synthesized) to produce the biologically active T_3. Vitamin A (retinoic acid) also has endocrine-like properties because its nuclear receptors are structurally homologous to those of steroid and thyroid hormones (Petkovich *et al.,* 1987). Vitamin A is essential to normal differentiation of epithelial structures, reproduction, vision, and immune system function (Zile and Cullum, 1983). Our result which showed that concentration of vitamin A in treated rats were maintained within the concentration range obtained in rats not exposed to the formulated diet, indicates that the administered extracts may have contributed in maintaining the metabolic and physiological integrity of organs and systems responsible for maintaining vitamin A homeostasis. This is so, because an underactive thyroid gland cannot efficiently convert dietary carotene to usable vitamin A.

The significantly decreased concentration of vitamin C in rats fed formulated diet only may affect transport of I^- using Na^+- K^+ ATPase into the follicular cell. Also, the integrity of thyroid gland may be affected because thyroid gland transport system is maintained by adequate vitamin C supply. The reduced vitamin C concentration observed in rats fed formulated diet only, may affect the concentration of the metabolically active hormones because vitamin C enhances the conversion of thyroid hormones. This corroborates the low thyroid hormones observed in this group. The significantly high concentration of vitamin C and normal thyroid hormone status in rats administered the extracts, may indicate structurally and physiologically maintained thyroid glands. Vitamin C functions effectively as antioxidant in the presence of vitamin E (α-tocopherol) to maintain organs and systems (Bender, 2003). Vitamin C reduces tocopheroxyl radicals (a product of α-tocopherol and lipid peroxide radical) to α-tocopherol (Metin, 2002).

Vitamin E possesses antioxidant properties that protect cells of living organisms against damaging effects of free radicals generated from metabolic and environmental activities. The use up of available vitamin E in antioxidative activities may be attributed to the reduced concentration of vitamin E in rats fed formulated diet only when compared with control. Living systems with adequate concentrations of vitamin E, will suffer less from the oxidative stress resulting from hypothyroidism. This is in line with the studies that showed protection from thyroid cell damage (Sarandöl *et al.,* 2005) and replication in thyroid cell in hypothyroid conditions (Oner *et al*., 2003). The administration of the extracts concomitantly with the formulated diet may have maintained vitamin E concentrations within the control rats' values, indicating reduced usage and/or damage to vitamin E. The flavonoid and lycopene contents of the extracts may have boosted the antioxidant status of the rats, because flavonoids can replace vitamin E as chain-breaking antioxidants in liver microsomal membranes (Dlipkumar and Preeti, 2013). These processes will lower the levels of reactive oxidative species (ROS), protecting thyroid membranes from possible oxidative damage (Fairfield and Fletcher, 2002).

As vitamins C and E function as antioxidants, vitamin D functions as a regulator of mineral metabolism (Bender, 2003). The significantly reduced concentration of vitamin D observed in rats fed formulated diet only may reduce calcium uptake by the bones (Faibish and Boskey 2005), with a consequential increase in serum calcium levels (Tryland *et al.,* 2006). Generally, vitamin D maintains calcium and phosphorous blood levels, and it is important in the absorption of calcium for proper formation and maintenance of strong bones. Goswami *et al*. (2009) reported that vitamin D deficiency may increase risk of thyroid autoimmune disease. Vitamin D deficiency has been shown to be more prevalent in patients with thyroid cancer or thyroid nodules relative to normal individuals (Laney *et al.,* 2010).

Conclusion

This study has shown the hormonal disruptive potential of roasted beef diet and its

tendencies to reduce tissue concentrations of some vitamins. However, the administrations of extracts of tomatoes and onions (used as sauce by locals in Nigeria) reversed these adverse effects, indicating chemoprotective potentials, which positively influenced thyroid physiology and the overall metabolism of thyroid hormones and vitamins. Since most Nigerians and other nationals, traditionally employ heat, flame and smoke from firewood as methods of food preparation and preservation, we encourage regular consumption of plant foods with proven antioxidants potentials and restraint should be applied in the consumption of roasted/smoked foods

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