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Thyroid hormone (T_3 and T_4) deficiency on the metabolism of vitamin A

Jahnabi Goswami[1], Umesh C.Goswami[2]
Department of Zoology
DST-FIST & UGC-SAP Sponsored Department
Gauhati University, Guwahati 781014, Assam, India

ABSTRACT: *Both cabbage and thiouracil exert high impact on thyroid hormone. In the present study it is found that decreasing amount of thyroid hormone (T_3&T_4) inhibit the metabolism of vitamin A in the freshwater fish Ctepharyngodon idella. The goal of this study was to determine the effect of deficiency of thyroid hormone on the retinoid reserve of the fish due to the administration of cabbage and thiouracil as feed. For this experiment fish Ctepharyngodon idella was kept in different feeding states. First was kept with aquatic weed hydrilla, mass grass, filamentous algae. Second group was kept with chopped cabbage and third group was feed with chopped cabbage along with thiouracil. Blood samples were collected from the caudal vasculature in the heparin zed syringes and plasma was assayed for plasma T_3&T_4 concentration by Radioimmunoassay(RIA). The vitamin A concentration i.e., retinol, dehydroretinol and β-carotene are estimated from the liver of the fish through HPLC procedure. The result shows concentration of mean plasma thyroid hormone was significantly decreased in fishes subjected to cabbage and thiouracil in their feed compared to those of fishes in controlled condition. It is also seen that retinol, dehydroretinol and β-carotene concentration also decreased to a considerable amount in fishes subjected to cabbage and thiouracil in their feed. So, from the present experiment, it is clear that thyroid deficiency can affect the retinoids reserves of fish.*

KEYWORDS*—Retinol, Dehydroretinol, β-carotene,T_3,T_4, Thiouracil, Cabbage*

I. INTRODUCTION

Vitamin A is a class of lipid molecules comprising of retinol, known as vitamin A_1 and dehydroretinol known as vitamin A_2 and carotenoids (having both provitamin A – status and others without provitamin A-activities) molecules existing in various organisms belonging to both the animals and the plant kingdoms [1,2]. Fish liver constitutes the most important natural source of vitamin A. The beneficial activity of animal liver, and fish liver in particular, has for many years earned a high reputation as a therapeutic product in curing some occular manifestations like night blindness and other nutritional deficiency diseases [2,3].

Various factors such as hormones, nervous control, nutritional status, overall physiological responses and environmental factors control the retinoids reserve as well as its synthesis [4,5,6]. Besides these, changes of season, presence of xenobiotics, age, sex and geographical isolation are responsible for the occurance, physiological actions of retinoids as well as pigmentation [4].

Several nutrients play important roles in the deposition as well as conversion of carotenoids into either retinol or Dehydroretinol [4].

In all vertebrates the thyroid gland is genetically programmed as the metabolic regulator. As metabolic regulators, the thyroid hormones exert numerous effects on nearly all tissues of the body in every vertebrate so far investigated. They accelerate cellular reaction in most tissues of the body, with some exceptions, and are well known for their calorigenic effect in homeotherms. But in poikilotherms this effect of thyroid hormone is less studied [7].

In the present experiments attempt has been made to see the effect of thyroid hormone(with reference to T_3 and T_4) on the metabolism of retinoids in the fish species *Ctenopharyngodon idella*, a herbivorous, surface feeder cyprinid fish. The species is widely used in culture system in South East Asian countries and India in particular. It is used along with major Indian carps in composite fish culture or mixed species culture system (*Labeo rohita, Catla catla, C.mrigala, H. molitrix*). Considering its herbivorous habit, being a voracious plant/ grass eater, the species is taken as an experimental model using cabbage as feed of the fish. Iodine is an important component of the aquatic environment as well as the plankton which are the fish food organism available in the habitant of the fish [1,4]. Use of iodine has problems. As the molecule sublimate directly, hence in the present studies T_3 and T_4 the thyroid hormone and thiouracil (a potent inhibitor of T_3 and T_4 or as whole on various metabolism of iodine), have been taken as a nutrient model to study any effects on the absorption as well as its metabolism.

Cabbage contains thiouracil [8]. In the present experiment, the fish become iodine deficient after feeding cabbage for a period of 45-60 days. The iodine deficiency in turn inhibits production of thyroid hormone. The same iodine deficient fish is used for different studies relating to metabolism of carotenoids with reference to biogenesis of either retinol and dehydroretinol.

In the present study, an attempt has been made to evaluate the role of thyroid hormone through T_3 and T_4 in the deposition as well as conversion of carotenoids in to vitamin A. Further the selection of grass carp (*C. idella*), a herbivorous fish which is reared on a cabbage administered diet is used in the depletion of T_3 and T_4 hormone from its blood plasma.

II. MATERIAL AND METHODS

***Fish**:*

Metabolism of carotenoids with reference to β-carotene, has been studied in grass carp, *Ctenopharyngodon idella*. Fingerlings of grass carp 10-15 cm, 25-30 g size and weight has been taken for conditioning the same into thyroid hormones (T_3 & T_4) deficient condition. The fishes were maintained with municipal water supply in glass aquarium (80 cm ×45 cm ×40 cm size, water depth 30 cm) and acclimatized for a week with the supply of various aquatic weeds (Hydrilla, Musk-grass, Naiads, Duckweeds, Pondweeds, Water pennywort, Filamentous algae, Water hyacinth etc) and later shifted to cabbage fed condition. The fishes were maintained in the following feed models

1st sets: Same Size grass carps were taken from the cultuted pond and reared with aquatic weed-hydrilla, mass grass, filamentous algae, 10g/6 h/60d. The aquatic weed are given during day time (12 h/day) and taken as control feed.

2nd sets: In this sets (n=3) of experiment fishes are administered with chopped cabbage 50g /6h hours feeding was maintained. As the fish do not consume in night, hence only 12 hours feeding starting from 6 am to 6 pm has been followed for 45-50 days in 3 aquarium keeping 5 fish in each.

3rd sets: In this sets, fishes were fed with chopped cabbage along with thiouracil (1g/kg cabbage /day through stomach tubis. The thiouracil was dissolved in water (1g in 10ml distilled water) and fishes were fed every alternate day.

Control Fish: *Ctenopharyngodon idella* of same size and age were selected for the cultured ponds, that has been reared along with major India carps,C*yprinus carpio* and *H. mollitrix* in the composite culture farm. Care has been taken to collect the fishes of same size and weight from the same farm.

Solvents and chemicals:

T_3 and T_4 are procured from Sigma Chemicals. RIA Kit was obtained from Bhaba Atomic Research Centre, Trombay, Mumbai, India. Thiouracil is procured from Sigma Chemicals. Light petroleum ether (b.p. 40-600 C, 60-80oC), L.R. grade was obtained from British Drug Houses (India), Glaxo Laboratories (India) Ltd. The solvent was dried over pure calcium chloride and distilled twice before used. Diethyl ether was supplied by Alembic Chemicals Works Co. Pvt. Ltd., Boroda, Gujrat, India. It was made peroxide free by distilling it over reduced iron. Chloroform was supplied from B.D.H. Chemicals Division, Glaxo Laboratories (India). Absolute ethanol was procured from Bengal Chemical and Pharmaceutical Works Ltd. Other solvents like acetone, acetic anhydride used were obtained from BDH, Laboratory Chemicals Division, Glaxo Laboratories (India) Pvt. Ltd. Crystalline ß-carotene, retinyl acetate, retinoic acid were obtained from Hoffman La Roche, Basel, Switzerland, BASF, Germany and Roche Co. Ltd., India. Antimony trichloride reagent and Anhydrous sodium sulphate are procured from Merck Limited (formerly known as E Merck), Mumbai, India.

***Blood Sampling**:*

Fish were euthanized with MS222 (200 ppm/100 liter basin). Blood samples were then collected from the caudal vasculature in heparin zed syringes (to prevent blood coagulation and the separation of serum). The blood samples were kept on ice for up to 30 min and then, plasma was separated using centrifuge and frozen at 4^0C for further thyroid hormones analysis.

***Thyroid Hormones Analysis**:*

Radiommunoassays (RIAs) were performed using T_3 and T_4 (RIA s) kits (supplied from BARC-Mumbai and gamavounter to determine T_3 and T_4 levels in plasma), as previously described [9] and their concentrations were computed in ng/ml plasma and were expressed as means ±SEM.

Estimation and Extraction of Retinoids:

Extraction of carotenoids and vitamin A

Lipids from the livers and the whole body of the fish were extracted through light petroleum (40-60°C) ether extract using anhydrous sodium sulphate [10,11]. The extraction efficiency was tested by following a parallel method [12]. 200mg/lit of BHT was added to Folch solution or light petroleum either which acted as antioxidant. It was found that both light petroleum and Folch solution showed similar extraction efficiency. However, in the present study, light petroleum (40-60°c) is used and retinyl propionate and β -apo-81 -carotenoic acid ethyl ester (CAEE) are used as internal standards.

The liver oil was extracted with light petroleum until the extract was colourless and gave no colour with $SbCI_3$ reagent. The combined extracts were filtered and the solvent removed by distillation under reduced pressure at 40°C. The last traces of the solvent were removed in vacuo and the oil preserved until further used or saponified under reflux for 10 minutes with methanolic solution of KOH (10% wt/ vol.). Vitamin A was extracted thrice with peroxide-free diethyl ether. The etheral extract was freed from alkali, dried over anhydrous sodium sulphate and the solvent was removed by distillation under reduced pressure. The saponicate was either dissolved in known volume of light petroleum ether or in HPLC solvent for estimation.

Estimation

The carotenoids and vitamin A extracted were estimated using HPLC technique [13].

HPLC procedures

HPLC system (waters) with column 300mm x 3.9 mm Nova -Pak Cl8 (4 hm) and a Guard -Pak precolumn module (water 5) were used. Standard retinoids samples (5.0 ms) were dissolved in 100 ml toluene: methanol (1: 1) containing 500 mg BHT (butylated hydroxy toluene) litre for producing 50µg/ml standards. These standard stock solutions could be preserved at -20°C for 4 months. HPLC grade solvents, acetonitrile: dichloromethane : methanol: water: propionic acid (71:22:4:2:1, v/v) were used as mobile phase with the flow rate of 1.0 ml/minute in the first 10 minute run, detection of carotenoids pigments was performed at 450 nm and retinol in 352 and 326 nm.

III. RESULTS

The presence of thiouracil in diet or from natural source affect metabolism of thyroid hormones. Earlier several works studied the metabolism of β-carotene both *in vitro* and *in viv* [14 -19].

In the present experiment the metabolism of β-carotene and concentration of plasma thyroid level has been studied with the three sets of feeding models. *Ctenopharyngodon idella* maintained in feeding models are fed for 45- 60 days and feeding controlled diet, cabbage as well as thiouracil. The details of experimental sets and *in vivo* metabolism are shown.

The iodine (T_3 & T_4) and vitamin A status of *Ctenopharyngodon idella* maintained in different diets are shown in Table 1. The results are presented as mean ±SEM. In all cases, $P<0.05$ was considered to be statistically significant.

The iodine (T_3 and T_4) status of *Ctenopharyngodon idella* maintained in cabbage and thiouracil added diets are estimated. T_3 and T_4 has been estimated from the blood plasma, while retinoids reserve (retinol, dehydroretinol and β-carotene) are estimated from the livers of the control, cabbage and thiouracil added diets administered fishes through HPLC procedure. The procedure of estimation of the above parameters is explained in details.

From the present findings, it is clear that metabolism of retinoid is significantly related to the thyroid hormone concentration. It is found that the introduction of cabbage and thiouracil in the fish diet resulted in significant decrease in thyroid hormone i.e., T_3 and T_4 in the blood of the experimental fishes. The liver of the same fishes when examined for determining the concentration of retinoid content, it was found that in both cabbage and thiouracil feed, the concentration of retinoid decreased to a considerable amount comparing to that of the controlled conditioned fishes.

The findings of the first experiment are presented in Table 1. It was found that in controlled condition, the thyroid concentration was 5.2(±1.0) ng/ml of T_3 and 2.1(±0.5) ng/ml of T_4 respectively while in cabbage & thiouracil feed condition it was found as 2.5 ng/ml of T_3, 1.0 ng/ml of T_4 and 2.0 ng/ml T_3 and 0.5 ng/ml of T_4 respectively. In the same way it was found that with the decreasing level of thyroid, the retinoids reserve of the fish also decreased. In the experiment no 1, the retinol, dehydroretinol and β-carotene in the controlled condition were found as 414(µg/g), 265(µg/g) and 150(µg/g) respectively. In cabbage feed condition it is found as 310(µg/g) of retinol, 215(µg/g) of dehydro retinol and 135(µg/g) of β-carotene respectively while in thiouracil feed condition, it is found as 240(µg/g) of retinol, 150(µg/g) of dehydro retinol and 75(µg/g) of β-carotene respectively.

The findings of the second experiment are presented in Table 2. In the 2nd *in vivo* experiment, the fish samples were also fed with carotenoids mixed diet with ground nut oil for a week through intestinal bulb tubing methods. In the metabolites isolated from the liver of *C. idella* after 45 days of exposure, the mean value of β-carotene amount is found as 350 (±4.5)µg/g in control where as in cabbage feed and thiouracil feed it is found as 270(±3.1)µg/g and 210(±2.5)µg/g. The retinol concentration has been found as 435(±15)µg/g in control condition and 300(±3.5)µg/g and 250 (±3.7)µg/g in cabbage feed and thiouracil feed condition. The dehydroretinol concentration has been found as 300(±2.5)µg/g in control condition and 218(±1.5)µg/g and 115(±4.0)µg/g in cabbage feed and thiouracil feed condition. The unidentified concentration has been found as 250(±1.5)µg/g in control condition and 200(±1.0)µg/g and 210 (±2.5)µg/g in cabbage feed and thiouracil feed condition.

It is again found that the metabolites isolated after administration of β-carotene from the liver of *C. idella* after 60 days of exposure, the mean value of β-carotene amount is found as 435(±1.7)µg/g in control condition where as in cabbage feed and thiouracil feed condition, it is found as 290(±2.1)µg/g and 250(±3.1)µg/g. The retinol concentration has been found as 410(±15)µg/g in control condition and 275(±2.2)µg/g and 260(±1.7)µg/g in cabbage feed and thiouracil feed condition. The dehydroretinol concentration has been found as 325(±3.9)µg/g in control condition and 260(±3.7)µg/g and 170(±1.2)µg/g in cabbage feed and thiouracil feed condition. The unidentified compound concentration has been found as 300(±5)µg/g in control condition and 285(±4.0)µg/g and 310 (±17)µg/g in cabbage feed and thiouracil feed condition.

Table 1: T_3 and T_4, retinol and dehydroretinol status of *Ctenopharyngodon idella* maintained in different diets

Diets	Expt.No / No. of Fish	Iodine status (ng/ml plasma)		Retinoids (µg/g)		
		T_3 (ng/ml)	T_4 (ng/ml)	Retinol	Dehydro Retinol	β-carotene
Control	i)n=3	5.2(±1.0)	2.1(±0.5)	414±17	2 65±5	150±1.5
	ii)n=5	6.0(±0.5)	1.6(±0.5)	450±30	210±2.5	120±7.0
	iii)n=3	5.0(±0.5)	2.0(±0.5)	425±7	195±14	110±10
Cabbage feed fish	i)n=3	2.5(±0.5)	1.0(±0.2)	310±10	215±7	135±4.5
	ii)n=5	2.7(±0.5)	0.6(±0.1)	279±2.5	160±10	110±2.5
	iii)n=3	2.2(±0.5)	0.7(±0.2)	325±1.3	150±2.5	75±1.5
Thiouracil added fish	i)n=3	2.0(±0.5)	0.5(±0.02)	240±1.5	150±1.0	75±5
	ii)n=5	2.2(±0.2)	0.5(±0.02)	200±3	110±4.5	45±2.5
	iii)n=3	1.5(±0.5)	0.2(±0.01)	230±2.5	55±1.5	35±6.5

Results are mean value of its experiment for the fish as mentioned. The differences are stastically analyzed (t-test) and found significant ($P<0.05$) after comparing with the results of the controlled experiment.

Table 2: Isolation of metabolites after administration of β-carotene from the liver of *Ctenopharyngodon idella* maintained in different diets.

Expt. No No of fish and No. of days	Metabolites	Diets		
		Control	Cabbage feed	Thioracil feed
1/ (n=3)	β-carotene (µg/g)	350 (±4.5)	270(±31	210(±25)
(45 days)	Retinol (µg/g)	435(±15)	300(±35)	250 (±37
	Dehydroretinol(µg/g)	300(±25)	218(±15	115(±40)
	Unidentified compound	250(±15)	200(±10)	210(±25)
2(n=4)	β-carotene	435(±17)	290(±21)	250 (±31
60d	Retinol	410(±30)	275(±22)	260 (±17)
	Dehydroretinol	325(±39)	260(±37)	170 (±12)
	Unidentified	300(±5)	285(±40)	310(±17)

The results are the mean value (±SE) of different experiment are statisticaly signify ($P<0.05$)

IV. DISCUSSION

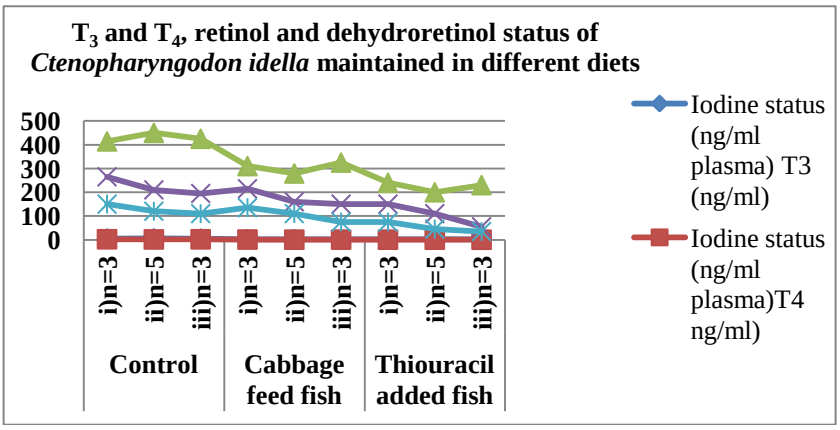
The aim of this present study was to investigate whether presence of cabbage and thiouracil in food has any effect in the decrease in the level of thyroid hormone in blood of fishes and how this decreased level of thyroid hormone impacts on metabolism of retinoids level of such experimental fishes. Cabbage is historically the plant in which a goitrogon property is attributed to the presence of antithyroid agents [20].

Thyroid hormone plays an important role in metabolism of fish. Thyroid regulation of metabolism in fish has been well recorded [21]. Thyroid hormones have a crucial role in the regulation of body weight [22]. Conflicting information has been gathered from investigations on the role of the thyroid hormone in lipid, glycogen, and protein regulation. It has been observed that radio-thyroidectomy results in increase in abdominal fat content in rainbow trout [23,24] and liver lipid content in platyfish [25]. It has been showed [26] that thyroxine treatment lowered liver and visceral lipid in brook trout. Otherwise, an increase in muscle lipid was noticed after the injection of thyroxine in coho salmon [27, 28].

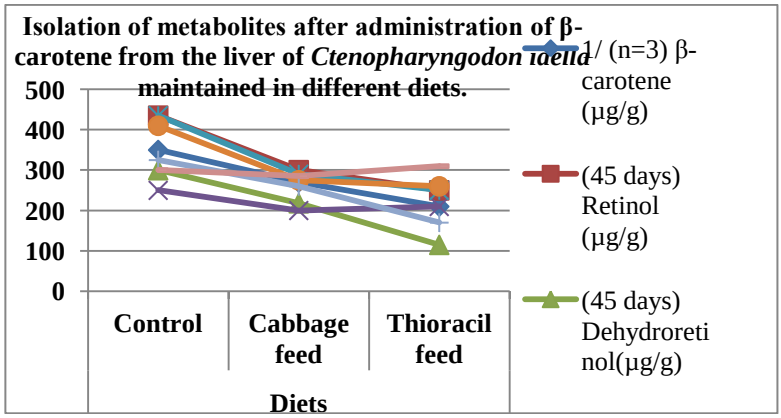
Thyroxine hormone administration has also been reported to increase the hepatosomatic index (HSI) in hypophysectomized goldfish [29].

In the present study the fish species *Ctenopharyngodon idella* is fed with cabbage and thiouracil which are thyroid deficient diet for two experiments. In the first experiment, the fish sample is kept for 45 days in the control, cabbage administered and thiouracil administered diet. The second experiment was conducted in two stages. In the first stage, the fish was kept for 45 days in control, cabbage and thiouracil administered diet. The fish sample was further administered with β-carotene. In the second stage another sample from the same source was kept for 60 days in the similar diet condition which was further administered with β-carotene.

Thereafter the status of T_3 and T_4 in blood, retinol and dehydroretinol and β-carotene in liver of the same fish were examined. The result from the first experiment has been tabulated in Table 1 and same has been graphically represented in Figure-1. It is seen that measure of T_3 & T_4, retinol and dehydroretinol in *Ctenopharyngodon idella* gradually reduces from control condition to cabbage feed condition and then further reduces to thiouracil feed conditions.



The measure of β-carotene (µg/g), Retinol (µg/g) ,Dehydroretinol(µg/g) and Unidentified compound found from both the stages of the second experiment has been tabulated in Table 2 and the same findings has been graphically represented in Figure 2. It is seen that measure of β-carotene (µg/g), retinol (µg/g) and dehydroretinol(µg/g) in *Ctenopharyngodon idella* gradually reduces from control condition to cabbage feed condition and then further reduces to thiouracial feed conditions.



It is also found [30] that mid (10µg/fish) and high (50 µg/fish) doses of thyroxine decreases the lipid content of liver and muscle. This finding is consistent with the data reported in *Salmo gairdneri* [31] and in Salvelinus fontinalis [26]. Increased adipose tissue lipase activity [26] and plasma free fatty acid levels [32] in response to the thyroxine treatment further substantiate the knowledge of fat mobilization from organ stores. However, the anabolic effect of thyroxine on lipid has been demonstrated in underyearling coho salmon [27] and yearling coho salmon [28].

So, from the present investigations, it is clear that presence of cabbage and thiouracil in fish food results in decrease in thyroid concentration in fish. Low thyroid concentration has significant impact on the deposition of carotenoids in fish body. The result is uniformity with the result found by the earlier investigations and it further substantiates their findings.

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