

Faecal Triiodothyronine Hormone Levels in Female Iguana during Weekday and Weekend in In Situ Conservation

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Abstract. Iguanas are exotic animals that are widely kept as pets in our society, their performance and color being their main attraction. Jogja Exotarium is an in situ conservation park that carries the back to nature concept. Animals that are kept at the Jogja Exotarium are allowed direct contact with visitors. This study aims to determine the hormone triiodothyronine in female iguanas that are raised and conserved in situ. This study used feces obtained from iguanas kept in Jogja Exotarium, sampling was carried out on Wednesdays and Sundays for 2 weeks. The collected faecal samples were processed by using freeze-drying method and then extracted using 80% methanol. The extracted samples were analyzed using the enzyme immunoassay (EIA) method. The results showed during the week day was 107.1 ± 7.01 ng/gr dry feces, while the week end was 119.95 ± 4.3 ng/gr dry feces. Based on the results of the study, it can be concluded that there is no difference in the hormone triiodothyronine in female iguanas during weekday and weekend

1 Introduction

Representatives of many families of lizards are commonly seen in the pet trade. The green iguana (*Iguana iguana*) is one of the most popular of all reptile pets, and historically has been the first reptile pet of many people. The green iguana is a tropical, arboreal, diurnal, somewhat communal (though not in captivity), generally non-seasonal, and non-hibernating lizard. Jogja Exotarium Minizoo is an ex situ conservation site that carries the theme of animal interaction with their visitors. The proximity of visitors and animals is expected to educate a sense of caring for animals. Zoo visitors with diverse behaviors can be a disruption to animals. When the animals are disturbed, there will be changes in their behavior and physiological conditions.

The thyroid gland exhibits both direct and indirect effects on the heart and cardiovascular system (i.e., influences on myocardial contractility and hemodynamics). The effects caused by thyroid hormones on the heart result from interaction with specific nuclear receptors in cardiac myocytes. Overall, changes in thyroid function influence cardiac action because T3 exerts a direct effect on cardiac myocytes by binding to nuclear T3 receptors; T3 may also influence the sympathetic system by increasing sensitivity, and T3 leads to hemodynamic alterations in the periphery that result in increased cardiac filling and modification of cardiac contractility. It has been suggested that clinical signs related to the

cardiovascular system are major manifestations of thyroid dysfunction in humans (1). A thyroid hormone receptor has been found in the testis of lizards (*Podarcis sicula*), which indicates that T3 might be involved in the regulation of gonadal activity. The investigators suggest that in lizards, the combined action of androgens, estrogen, and T3 might regulate testicular activity (2).

Several diagnostic tests associated with thyroid function have been developed over the years, and most are currently being used in veterinary medicine. The most common initial assessment of thyroid function is based on the measurement of serum thyroid hormones. Although with this method, the process of taking blood samples from animals that are not used to be handled might cause acute stress, leading to less accurate results (1). There are few validated, quantitative techniques for assessing reproduction, health and physiological stress levels non-invasively. Such techniques are essential to correctly interpret trends in reproduction and stress, forecast potential effects on populations, and identify and minimize human impacts. Feces typically contain high concentrations of immunoreactive metabolites of all five classes of steroid hormones (progesterone [fP], testosterone and related androgens [fT], estrogens [fE], glucocorticoids [fGC], and mineralocorticoids) as well as some thyroid hormones (fThy; metabolites of thyroxine and triiodothyronine) (3). Research needs to be done to scientifically explain the influence of visitors on animal physiology during weekday and weekend, especially the T3 hormone as an indicator of metabolic hormones.

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2 Materials And Methods

The materials used for this research includes faecal samples taken from a female green iguana (*Iguana iguana*) at Jogja Exotarium Mini Zoo, Sleman, Yogyakarta. The tools and equipment used in this study includes ballpoint pens/ markers, plastic clips, gloves, refrigerator/ freezer, plastic pot, conickel tubes, measuring spoon, scale, vortex, ependorf tubes, freeze dryer machine, ELISA washer, ELISA reader, dropper, micropipette, triiodothyronine competitive ELISA kit, 80% methanol, and labels.

2.1 Sample collection and preparation

The fecal samples of the female iguana were taken in the two weekday and weekend, than placed into a plastic clip and given labels for the date of collection was first kept in a freezer then used for analysis. For the preparation, the fecal samples was placed into freeze dried for 72 hours. After that, the samples were pulverized and separated from any debris to avoid contamination. The pulverized fecal samples were then taken and weighed for 5mg, then put into a conickel tube. 5 ml of 80% methanol was added and the mixture was homogenized using a vortex stirrer for 10 minutes. The samples were stored in the refridgerator for further analysis.

2.2 Sample analysis

The analysis of triiodothyronine (T3) hormone from feces was carried out using a triiodothyronine competitive ELISA kit. Firstly the antigen is bound by adding 25µl of standards or samples to the appropriate wells. 1x assay buffer is added into the wells for detecting non-specific binding (NSB). 50µl of triiodothyronine (T3) conjugate is added to each well. 50µl of triiodothyronine (T3) antibody is added to each well except NSB wells. The plate is then mixed and covered and left to incubate for 60 minutes. The solution is then aspirated and the wells are washed 3 times with 300µl of 1x wash buffer. Next the chromogen is added. 100µl TMB substrate is added to each well and incubated again. Then 50µl of stop solution is added to each well and the plate is mixed. The plate is then read after adding the stop solution.

3 Results and discussion

Faecal Triiodothyronine (T3) hormone level analysis in this research obtained from a female iguana specimen in Jogja Exotarium Mini Zoo was used to study whether the number of visitors at the site affected the particular hormone production where increased visitors during weekends might increase Triiodothyronine (T3) levels due to increased stress.

In general reptiles have plasma concentrations of thyroid hormones that are far less than mammals which has complicated thyroid hormone detection with assays designed for the higher levels of mammals. T4 and T3 values in reptiles are roughly 20 and 25%, respectively, of the average values in mammals. T4 has the highest

concentration, followed by T3, free T3 (fT3), and free T4 (fT4). It is believed T4 plays a more central role in thyroid metabolism. In experimental studies T4 accelerated shedding more than T3 (4)

The majority of T3 and T4, called total T3 (TT3) and total T4 (TT4), are bound to the plasma protein (i.e., the thyroxine-binding globulins transthyretin and thyroidbinding albumin). Thyroxine-binding globulins bind ~70% of plasma T4 and ~75% to 80% of T3. Only a small fraction of each is freely present in plasma; free thyroxine (fT4) accounts for 0.02% to 0.03% of TT4, while free triiodothyronine (fT3) accounts for ~0.3% of TT3 (1) Fecal hormone analysis is an alternative, noninvasive technique that is now widely employed for studies of stress and reproductive physiology in terrestrial wildlife. In most vertebrates, steroid and thyroid hormones are removed from circulation by the liver, with a portion excreted into bile and then into the gut lumen. Within the gut, a given steroid hormone is metabolized to a suite of various breakdown products ("fecal hormone metabolites", FHM) which are eventually excreted in feces (3)

Table 1. Average of triiodothyronine level of Iguna in the weekday and wek end

	level T3 ng/gr dry faecal
weekday	107.1 ± 7.01
weekend	119.95 ± 4.3

Analyze statistic shown that, triiodothyronine level does not different significantly between weekday and weekend. In the weekday, number of visitor more less than in the weekend but based on the Table 1, it can be seen that the number of visitors do not affect the T3 hormone levels. One reason that might explain this is that the animal is already accustomed to the number of visitors and without a huge spike, it will not show much variability. Reptiles are highly adaptable and due to the enclosure set up, there is not much interaction between the visitors and the female iguana which the sample was taken from. In addition, based on other previous studies from (5), thyroid hormones are particularly responsive to nutritional deficits, lowering metabolism and allowing the body to conserve energy during a nutritional emergency but appear unaffected by psychological stress. By contrast, glucocorticoid (GC) measures increase in circulation and feces in response to both psychological and nutritional stress. It may be due to this that there is not much correlation between the number of visitors and level of triiodothyronine (T3) level fluctuation.

Thyroid activity is regulated by a negative feedback loop involving the hypothalamus, pituitary, and thyroid gland. TRH is secreted by the hypothalamus after exposure to stress, illness, cold, metabolic demand, or decreased circulating thyroid hormones, mainly T3. TRH stimulates the pituitary to release TSH, which causes the release of T3 and T4 (6). Other conditions that can affect this axis include drugs, illness, thyroid disease, pituitary

disorders, and age. Thyroid hormones have a negative effect, which is the primary TSH regulatory mechanism, although tonic stimulation of TRH has a permissive role in TSH secretion. It is also suggested that thyroid hormones have a direct negative effect on TRH release from the hypothalamus (1).

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