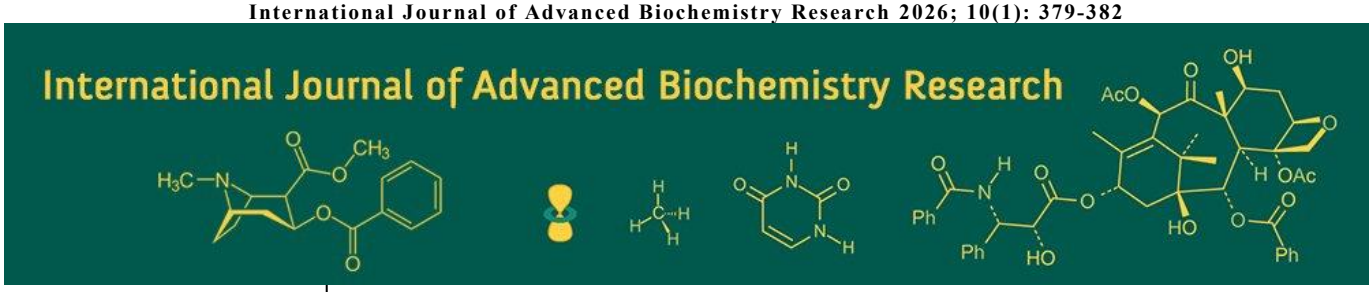

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Reference levels of serum thyroid hormones profile in Osmanabadi bucks

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Abstract
Present study was undertaken to establish the reference range of thyroid hormones *viz*. Total triiodothyronine (TT_3), Total thyroxine (TT_4) and free Thyroxine (fT_4) in Osmanabadi bucks. Total 226 blood samples were collected from healthy adult Osmanabadi bucks from Advy Chemicals Pvt. Ltd. farm located at Phalegaon, Kalyan, Dist. Thane (India). Serum samples were analyzed for thyroid hormones using radioimmunoassay (RIA). Reference ranges for hormone concentrations were established by the non-parametric method of percentile estimates (25th to 75th percentile). The mean value of T_3 of Osmanabadi bucks was 103.15±2.54ng/dl. The box plot of TT_3 concentration in goats where, 'T bars' represent the range from 19.5 to 201ng/dl, interquartile range (25th to 75th percentile), as 76.6 to 127.1ng/dl and median 97.68ng/dl. The mean value of TT_4 of healthy Osmanabadi bucks was recorded as 5.43±0.15µg/dl. The box plot of TT_4 concentration in goats where 'T bars' represent the range from 1.18 to 11.2µg/dl, interquartile range (25th to 75th percentile), as 3.8 to 6.9 µg/dl and median as 4.9µg/dl. The mean value of fT_4 of healthy Osmanabadi bucks was recorded as 0.99±0.01ng/dl. The box plot of fT_4 concentration in goats where 'T bars' represent the range from 0.46 to 1.58ng/dl, interquartile range (25th to 75th percentile), as 0.85 to 1.84ng/dl and median as 1.0ng/dl. The established reference range for blood serum levels of TT_3, TT_4 & fT_4 are 76.6 to 127.1ng/dl, 1.18 to 11.2µg/dl and 0.85 to 1.84ng/dl respectively in Osmanabadi bucks. The physiological serum thyroid hormones reference range levels would be valuable information for identifying thyroid dysfunction in Osmanabadi bucks.

Keywords: Triiodothyronine, thyroxine, free thyroxine, Osmanabadi bucks, radioimmunoassay

Introduction
Thyroid hormones (THs) play a pivotal role in the regulation of metabolic activity throughout all life stages (Behringera *et al*., 2018) [1]. Appropriate thyroid gland function and activity of thyroid hormones (TH) are considered crucial to sustain the productive performance in domestic animals (growth, milk, hair fibre production) and circulating thyroid hormones can be considered as indicators of the metabolic and nutritional status of the animals (Riis and Madsen, 1985; Todini *et al*., 2007) [7, 9]. Osmanabadi goats is one of the most popular breeds of goat amongst the livestock in Maharashtra, India. The Osmanabadi goats particularly males are being used for meat purposes and also, in industry for raising various antisera production as an experimental animal.

There are very few references available for the normal physiological range of thyroid hormones *viz*. T_3, T_4 and for fT_4 no reports are available for male goats. Thyroid hormones are affected by various factors, *viz*. Season, climate, geographical distribution, age, sex, nutritional status and disease conditions (Todini, 2007) [9]. Radioimmunoassay (RIA) is a sensitive *in vitro* technique for assessing antigens from biological fluids with excellent sensitivity and precision (Goldsmith, 1975) [4]. The purpose of the present study was to establish thyroid hormones reference range in healthy Osmanabadi buck using RIA technique.

Collection and preservation of blood samples
The Advy Chemicals Pvt. Ltd. farm located at Phalegaon, Kalyan, Dist. Thane (India) having more than 500 Osmanabadi buck. Those animals are being maintained on standard managemental conditions. The company replace the healthy male goat lot periodically and being purchased from farmers in Nasik, Ahmednagar district area of Maharashtra state.

The goats are being used for the raising antisera against various animal and human diseases. To established the normal reference range of these thyroid profile, total 226 blood samples (3-5 ml) were collected from adult Osmanabadi bucks with age range 1-1.5 year and average body weight 28.5 kg from Advy Chemicals Pvt. Ltd. farm located at Phalegaon, Kalyan, Dist. Thane (India). The clear serum was obtained immediately after collection by centrifugation and stored at -20ºC till to be analyzed.

Assay of Thyroid Hormones

Total 226 Serum samples were analyzed for thyroid hormones *viz*. total triiodothyronine (T_3), thyroxine (T_4) and fT_4. Radioimmunoassay was conducted by implying ready to use kits supplied by Board of Radioisotope Technology (BRIT), Bhaba Atomic Research Centre. The radioimmunoassay was carried out at Radiation Medicine Centre (RMC), BARC, Parel, Mumbai - 400 012.

All pre-analytical, analytical and post analytical precautions were taken into consideration for ensuring proper quality. All Standard Operating Procedures (SOPs) were followed for sample collection, processing, storage and handling. Internal quality control (QC) was done for each parameter by using lyophilized Quality Control. Intra- assay and inter-assay coefficient of variance was carried out. The reference ranges were considered only after verification of control ranges. Westgard rules were followed to ensure quality.

Statistical analysis: The experimental data was tabulated and reference ranges for hormone concentrations were established by the non-parametric method of percentile estimates (25th to 75th percentile) (Snedecor and Cochran, 1994) [8].

Results & Discussion

As depicted in box plot Fig:1, the mean value of T_3 of Osmanabadi bucks was 103.15±2.54ng/dl. 'T bars' represent the range from 19.5 to 201ng/dl, interquartile range, 25th to 75th percentile, as 76.6 to 127.1ng/dl and median 97.68ng/dl. The mean value of TT_4 of healthy Osmanabadi bucks was recorded as 5.43±0.15µg/dl (69.9nmol/L). The box plot (Fig.2) of TT_4 concentration in goats where 'T bars' represent the range from 1.18 to 11.2µg/dl, interquartile range, 25th to 75th percentile, as 3.8 to 6.9 µg/dl and median as 4.9µg/dl. The mean value of fT_4 of healthy Osmanabadi bucks was recorded as 0.99±0.01ng/dl. The box plot (Fig.3) of fT_4 concentration in goats where 'T bars' represent the range from 0.46 to 1.58ng/dl, interquartile range, 25th to 75th percentile, as 0.85 to 1.84ng/dl and median as 1.0ng/dl. The reference range for blood serum levels of TT_3, TT_4 & fT_4 are 76.6 to 127.1ng/dl, 1.18 to 11.2µg/dl and 0.85 to 1.84ng/dl respectively in Osmanabadi bucks. The mean and interquartile reference range of TT_3 levels in our studies are slightly lower than the values reported by Galdhar *et al* (2022) [5] in different breeds of adult goats (n=102) with RIA technique. They reported mean total T_3 levels of 2.42±0.09nmol/L (157ng/dl) with reference interval (25th to 75th percentile) 1.69 to 2.98nmol/L. Mean total T_4 levels of 92.63± 3.63 nmol/L (7.2µg/dl) with reference interval (25th to 75th percentile) was 64.67 to 111.64nmol/L.

In adult sheep more than 99.9% of T_4 and 99.5% of T_3 circulate in blood bound to plasma proteins (Chopra *et al*., 1975) [2]. Only the free hormone is responsible for the biological activity. Few reports are available for the levels free T_4 in goats. No systematic studies with sizable sample size have been carried out in goat. The levels of fT_4 reference range have been established first time in goat in our study. The levels of fT_4 observed in our study are in close agreement with the reports of I.R. Dias *et al*., (2010) in Churra -da -Terra- Quente ewes from north east Portugal. They reported mean value of 11.93± 1.78pmol/L (0.92±ng/dl). Also, range for total T_3, T_4 and fT_4 reported by Luigi Liotta *et.al.,* (2021) [6] in nonpregnant Nicastrese goats are in close agreement with our findings in our studies in Osmanabadi buck without much difference in the reference range in adult male and female animals.

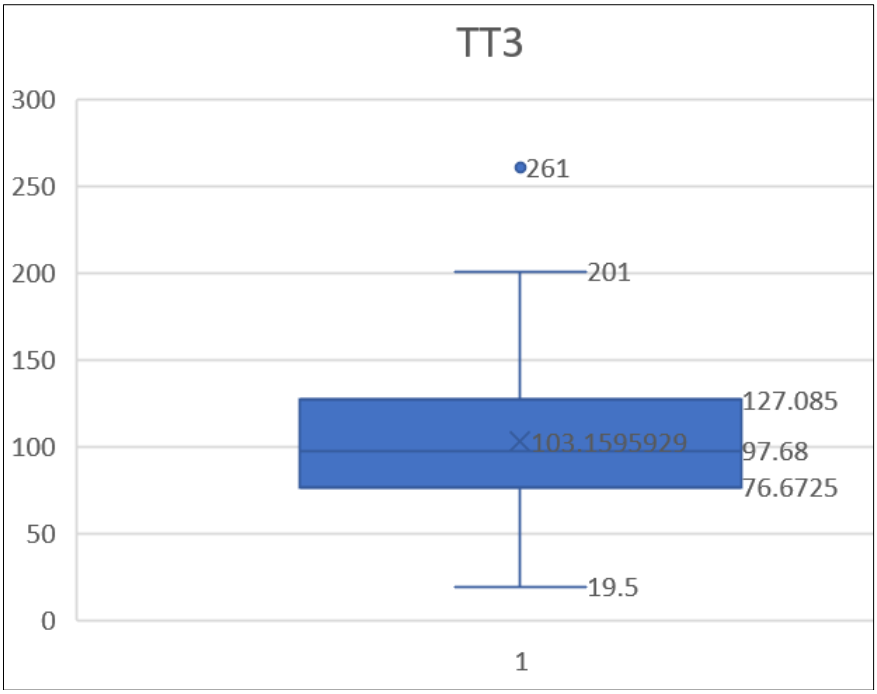

Fig 1: Box plot depicting Triiodothyronine concentration in Osmanabadi bucks(n=226)

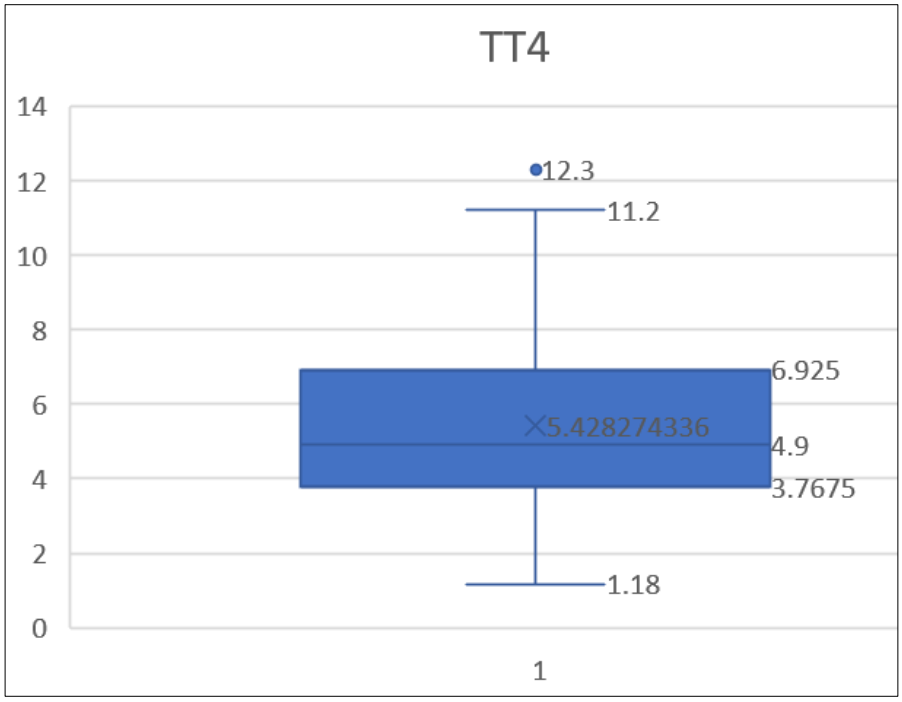

Fig 2: Box plot depicting thyroxine concentration in Osmanabadi bucks(n=226)

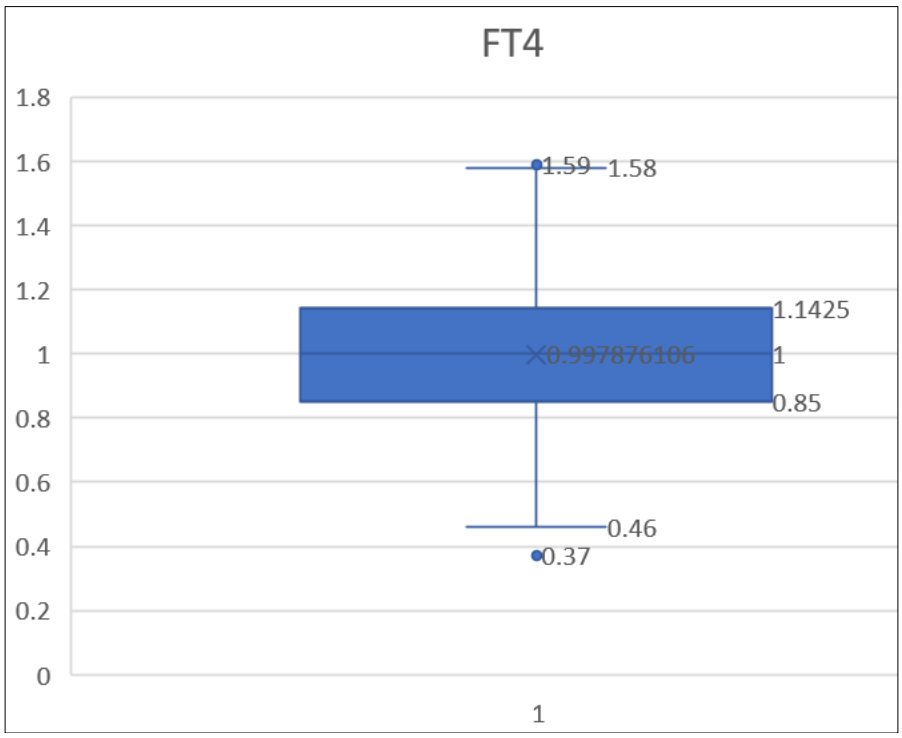

Fig 3: Box plot depicting fT_4 concentration in Osmanabadi bucks(n=226)

Summery & Conclusion

Serum total triiodothyronine levels range from 76.6 to 127ng/dl, total thyroxine concentration range from 3.8 to 6.9 µg/dl and fT_4 concentration in goats range from 0.85 to 1.84ng/dl Osmanabadi bucks. This physiological serum thyroid hormone profile reference range levels would be valuable information for identifying thyroid dysfunction in Osmanabadi bucks. There were no systematic studies have been carried out on thyroid hormone levels in goat and the levels of Free T_4 (fT_4) established first time in goat, which can be important biomarker with narrow reference range to study the thyroid status in this animal.

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Conflict of interest

There are no conflicts of interest to declare by any of the authors. The animal study protocol was approved by the Institutional Animal Ethical Committee (IAEC) of college.

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