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Research Articles

Radio immune assay (RIA) enabled thyroid profile (TT_3, TT_4 and fT_4) in pigs

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Abstract

Present study was undertaken on 100 clinically healthy pigs to establish reference range of Total tri-iodothyronine (TT_3), Total Thyroxine (TT_4), and free thyroxine (fT_4) by radio immune assay (RIA). Healthy pigs were enrolled in the study and assessed for their health on clinical data, hematological and biochemical profile after statutory approval from Mumbai Veterinary College institutional ethical committee. The mean serum concentration TT_3 (nmol/l), TT_4 (nmol/l) and fT_4 (pmol/l) in apparently healthy pigs were 0.81±0.04 (range, 0.15-2.30); 41.58±1.23 (range, 17.37-70.79) and 5.31±0.18 (range:- 2.57-10.81) respectively. A significant difference ($p\leq0.05$) was recorded in thyroid profile of pigs according to their age, sex, breed and diet. Age wise thyroid profile revealed significant difference ($p\leq0.05$) in TT_4 and fT_4 and non-significant ($p\leq0.05$) difference in TT3. Sex wise thyroid profile revealed significant ($p\leq0.05$) difference in male and female TT_3 and fT_4 and non-significant ($p\leq0.05$) difference in TT_4. Breed wise, thyroid profile revealed significant ($p\leq0.05$) difference in TT_3, TT_4 and fT_4. Significantly ($p\leq0.05$) higher concentration of TT_3 and fT_4 was recorded in pigs fed on kitchen waste than pigs offered commercial diet.

Keywords: Radio immune assay (RIA), Thyroid Profile, Pig, TT_4, TT_3, fT_4

Thyroid hormones (TT_3, TT_4 and fT4) in canines are produced by thyroid glands, which is structurally similar with human beings. These hormones are vitally important for various metabolic activities. Calorigenic thyroid hormones are important in fetal life specifically for development of neural and skeletal system. In short, thyroid hormones deficiency or excess affects all systems in the body. Thyroid hormones are iodine containing amino acids synthesized in the thyroid glands. Thyroglobulin is formed in follicular cells and is secreted into colloid. Iodine is oxidized and bound to thyroglobulin and form monoiodothyronine and diiodothyronine which then undergo oxidative condensation to form T_3 and T_4. The production and release of thyroid hormones are controlled via negative feedback mechanism. Pigs have unique anatomical difference as they have accessory thyroid tissue and as much as six accessory glands in one animal (Caylor and Schlotthauer, 1927; Gaikwad, 2005).

Radio Immune Assay (RIA) is an extremely sensitive *in vitro* assay technique to measure concentrations of antigen viz. hormones in biological fluids using antibodies. The technique is impressively sensitive and specific with the ability to measure small molecules. I^{125} is the radioisotope used in RIA procedures. The method offers convenient assay of large numbers of samples with good precision. It is able to assay materials that are difficult to detect and measure using other techniques (Goldsmith, 1975). Since 1970, technological advances in RIA have progressively improved the specificity, reproducibility and sensitivity of thyroid testing methods and thus RIA has been the method of choice for measuring circulating levels of thyroid hormones in vertebrates (Noyes *et al*, 2014). Further, RIA is considered as the best assay to estimate hormones as compared to other techniques viz. ELISA and CLIA (Chemiluminescence Immunoassay). RIA technique is sensitive, cost effective and the use of long half-life radioactive materials (I^{125}; physical half-life 60 days) facilitates its use for long time (Galdhar and Gaikwad, 2017).

Mumbai Veterinary College has established a dedicated Veterinary Nuclear Medicine facility comprising i*n vivo* diagnostic facility (Gamma Scintigraphy) and *in vitro* diagnostic facility (Radio Isotope laboratory). The Radio Isotope laboratory established thyroid profile reference range for companion animals in India (Naik, 2016; and Dadke, 2018). The literature about reference range of thyroid profile by RIA in pigs in India is not only limited but also unavailable in clinical setup to diagnose porcine thyroid dysfunction. Therefore, present study was planned with an objective to measure thyroid hormone concentration (TT_4, TT_3 and fT_4) in healthy pigs by RIA and establish its reference range for pigs in India. This established reference range may have direct application in medical diagnosis, therapeutics and research.

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Material and Methods

Statutory permissions: The present study was approved by Institutional Ethics Committee for Veterinary clinical research (Project Approval No: IAEC-VCR/ Subcommittee/07/2018) and Institutional Bio-safety Committee (IBSC) meeting held on 25.04.2019 at Mumbai Veterinary College, Mumbai.

Selection of Animals: Total one hundred (n=100) pigs were selected over a period February to August, 2019. All pigs were enrolled after permission from competent authority of host institution and private farm owners from where pigs were selected. The pigs included in the study were from Krantisinh Nana Patil College of Veterinary Science (Shirwal-M.S.), National Research Center on Pig (Indian Council of Agricultural Research, Rani, Near Airport, Guwahati- 781 131, All India Co-ordinated Research Project on Pig (College of Veterinary Science, Assam Agricultural University, Khanpara, Guwahati-781022), Instructional Livestock Farm Complex (College of Veterinary Science, AAU, Khanapara, Guwahati) and Private commercial farm (Veti), Kasa, Palghar district (M.S.).The data about age, sex, breed and diet were collected for enrolled pigs.

Anamnesis, Clinical Examination and Hemato-biochemical Profile: Healthy pigs were assessed on the basis of anamnesis, routine clinical examination, and evaluation of hemato-biochemical profile. During enrollment of these healthy pigs in the present investigation, anamnesis about appetite, diet, deworming, vaccination and illness was recorded. Blood samples were collected and subjected for routine hematological and biochemical evaluation as per the standard methods. The results of hematological and biochemical profile of enrolled animals is presented in Table 1 and 2 respectively.

Thyroid Profile (hormonal) Assay: Concentration of $TT_{3,}$ TT4 and fT_4 in serum samples was determined by using ready to use RIA kits supplied by Board of Radiation and Isotope Technology (BRIT) Mumbai. The assay procedure for estimation of TT_3, TT_4 and fT_4 was followed as per methods described by manufacturer. Results of hormonal concentration were extracted from standard graph after satisfactory quality control parameters like observed value of controls supplied with kits and recovery percentage.

Statistical Analysis: Mean and standard error of collected data was calculated and analyzed for comparison as per standard methods outlined by Snedecor and Cochran (2009) .

Results and Discussion

Thyroid profile in healthy pigs: Mean concentration of TT_3, TT_4 and fT_4 of healthy pigs (n=100) is presented in Table 3.

TT3: The mean TT_3 concentration and range recorded in the present research work was 0.81 ± 0.04 nmol/l and 0.15-2.30 nmol/l, respectively. This estimated magnitude of mean value of TT_3 (0.81±0.04 nmol/l)

Table 1: Mean values of hematological profile in healthy pigs (n=100)

Sr. No.	Parameter	Mean ± S.E.	Reference Range (Rahman, 2016)
1	Hb (gm %)	10.35 ± 0.30	10-16
2	PCV (%)	29.42 ± 0.97	32-50
3	TEC (X 10^6/cmm)	5.68 ± 0.17	5-8
4	TLC(X 10^3/cmm)	22.12 ± 0.80	11-22
5	DLC (%)		
	N	50.48 ±1.34	28-47
	L	42.03 ±1.32	39-62
	M	5.11 ±0.09	2-10
	E	1.88 ±0.07	0.5-11
	B	0.34 ±0.02	0-2
6	MCV (fl)	51.56 ± 0.35	50-68
7	MCH (pg)	18.15 ± 0.17	17-21
8	MCHC (gm/dl)	35.43 ± 0.33	30-34
9	PLT(lacks/cu mm)	672070 ± 58940	200000-800000

Table 2: Mean values of biochemical profile in healthy pigs (n=100)

Sr. No.	Parameter	Mean ± S.E.	Reference Range (Rahman, 2016)
1	Blood Urea Nitrogen (BUN, mg/dl)	11.32±0.37	4.7-12.6
2	Creatinine (mg/dl)	1.02±0.04	0.8-1.8
3	Total Bilirubin (mg/dl)	0.51±0.02	0-3.4
4	Direct Bilirubin (mg/dl)	0.27±0.01	0-1.7
5	Indirect Bilirubin (mg/dl)	0.25±0.01	0-1.7
6	Alkaline Phosphatase (ALP, U/L)	201.84±15.11	0-300
7	Aspartate transaminase (AST, IU/L)	42.48±3.03	0-125
8	Alanine transaminase (ALT, IU/L)	66.80±3.06	0-103
9	Total Protein (gm/dl)	5.88±0.13	4.9-6.7
10	Albumin (gm/dl)	2.66±0.08	1.9-2.9
11	Globulin (gm/dl)	3.21±0.09	2.8-4.1
12	Serum Cholesterol (mg/dl)	76.80±1.13	77-1.62
13	Serum Triglyceride (mg/dl)	53.27±4.73	26-239
14	Random Blood Glucose (mg/dl)	76.79±1.44	77-154

Table 3: Mean values of thyroid profile in healthy pigs (n =100)

Sr. No.	Name of Parameter	Mean ± SE (Range)
1	TT3 (nmol/l)	0.81 ± 0.04 (0.15-2.30)
2	TT4 (nmol/l)	41.58 ± 1.23 (17.37-70.79)
3	fT4(pmol/l)	5.31 ± 0.18 (2.57-10.81)

is in accordance with internationally published data documented by Eder and Stangl (2000), Zhan *et al*. (2006), Chakraborty *et al.* (2017) and Pathak *et al.* (2018).

TT4: The mean TT_4 concentration and range recorded in the present research work was 41.58±1.23 nmol/l and 17.37-70.79 nmol/l, respectively. This estimated magnitude of mean value of TT_4 (41.58±1.23 nmol/l) is in accordance with internationally published data documented by Armstrong *et al.* (2001), Svetina *et al.* (2003), Chakraborty *et al.* (2017) and Pathak (2018).

fT4: The mean fT_4 concentration and range estimated in present research work was 5.31±0.18 pmol/l and 2.57-10.81 pmol/l respectively. The mean value of fT_4 (5.31±0.18 pmol/l) recorded in present study is in accordance with ranges established by Zhan *et al.* (2006).

Age wise thyroid profile: Age wise deviations in mean concentration of thyroid profile are presented in Table 4. The mean concentration of TT_3 values for group 1, group 2 and group 3 were recorded as 0.94±0.05 nmol/l, 0.61±0.07 nmol/l and 0.54±0.15 nmol/l, respectively. Statistically non-significant ($p \leq 0.05$) difference was observed in TT_3 concentration in all three age groups of pigs, but a trend of gradual decrease in values of TT_3 was recorded as age increases.The mean concentration of TT_4 values for group 1, group 2 and group 3 were recorded as 45.42±1.31 nmol/l, 37.52±2.52 nmol/l and 28.96±4.40 nmol/l respectively. The group 1 showed statistically significant ($p \leq 0.05$) difference with group 3 but non-significant with group 2 in the concentration of TT_4 and age of pigs. It was observed that as the age of pig increases, the TT_4 concentration decreases gradually. The mean concentration of fT_4 values for group 1, group 2 and group 3 were recorded as 5.72±0.26 pmol/l, 4.95±0.18 pmol/l and 3.64±0.39 pmol/l respectively. The group 1 showed statistically non-significant ($p \leq 0.05$) difference with group 2 but showed significant ($p \leq 0.05$) difference with group 3 in fT_4 concentration. This suggested that fT4 value gradually decreases as age increases. About age-dependent study of changes in thyroid profile, in piglets, present study demonstrated decreased concentrations TT_3, TT_4 and fT_4 as age progresses. The highest mean concentrations of TT_3, TT_4 and fT_4 were observed in young piglets (age

0-12 months) and lowest in adult animals (>24 months of age). Iveta *et al.* (2011) reported highest mean concentrations of T_3 and T_4 in the youngest piglets. It is known that age related changes in metabolism of T_3 and T_4 may affect activity of thyroid hormones during development and ageing reduces conversion T_4 to T_3 in the liver and brain. Similarly in canines, Dadke (2018) reported a decreased trend in the thyroid profile as age advances.

Sex wise thyroid profile: Sex wise deviations in mean concentration of thyroid profile are presented in Table 5. The mean concentration of TT_3 in male pigs was 1.00±0.07 nmol/l and in female pigs was 0.69±0.05 nmol/l respectively. Significant ($p \leq 0.05$) difference was observed between TT_3 concentration in two genders. In present study, the male pigs population showed higher TT3 concentration than females. The mean concentration of TT_4 in male pigs was 41.75±1.69 nmol/l and in female pigs was 41.46±1.72 nmol/l respectively. Non-significant ($p \leq 0.05$) difference was seen in TT_4 concentration between males and females. The mean concentration of fT_4 in male pigs was 5.99±0.36 pmol/l and in female pigs was 4.85±0.15 pmol/l respectively. Statistically significant ($p \leq 0.05$) difference was seen between free thyroxin concentration in both gender groups. Where, male pigs population showed high fT_4 concentration than female pigs. Thyroid hormone levels tended to be sex influenced, with higher TT_3 and fT_4 concentrations found in males than females, whereas unchanged concentration recorded in TT_4 in the present study. This trend is in agreement with those reported in pigs by Petkov *et al.* (2008), who stated that, the influence of age and gender on thyroid function provides evidence that the parameters of thyroid hormone synthesis change together with the alterations in the other elements of the endocrine chain. In the present investigation, sex-biased thyroid hormone levels are comparable due to the differences in the metabolic physiology and reproduction mechanism between the female and the male. In mammalian species, thyroid hormones are essential for the maintenance of female reproductive behaviors (e.g. sustain pregnancy and raise offspring).

Breed wise thyroid profile: Breed wise deviations in mean concentration of thyroid profile are presented in Table 6. Mean TT_3 concentration recorded in Large White Yorkshire (LWY), Ghungroo, Hampshire × Desi × Khanapara (HDK75), Hampshire and Large White Yorkshire × Local (LWYL) were 0.76±0.08 nmol/l, 0.59±0.07 nmol/l, 0.96±0.07 nmol/l, 0.38±0.08 nmol/l and 1.19±0.07 nmol/l respectively. The Mean TT_4 concentration observed in LWY, Ghungroo, HDK75,

Table 4: Age wise deviation in thyroid profile in healthy pigs (n=100)

Sr. No	Parameter	Age Group	Concentration (Mean ± S.E)
1	TT_3 (nmol/l)	Group 1 (0-12 Months) (n=60)	0.94±0.05
		Group 2 (12-24 Months) (n=32)	0.61±0.07
		Group 3 (Above 24 Months) (n=08)	0.54±0.15
2	TT_4 (nmol/l)	Group 1 (0-12 Months) (n=60)	45.42±1.31^{a}
		Group 2 (12-24 Months) (n=32)	37.52±2.52^{ab}
		Group3 (Above 24 Months) (n=08)	28.96±4.40^{b}
3	fT_4(pmol/l)	Group 1 (0-12 Months) (n=60)	5.72±0.26^{a}
		Group 2 (12-24 Months) (n=32)	4.95±0.18^{ab}
		Group3 (Above 24 Months) (n=08)	3.64±0.39^{b}

Mean should be read column wise for comparison. Mean showing dissimilar superscript differ significantly ($p \leq 0.05$)

Table 5: Sex wise deviation in thyroid profile in healthy pigs (n=100)

Sr. No	Parameter	Sex	Concentration (Mean ± S.E)	t cal	t table (p<0.05)
1	TT_3 (nmol/l)	Male (40)	1.00 ± 0.07	3.86*	1.98
		Female (60)	0.69 ± 0.05		
2	TT_4 (nmol/l)	Male (40)	41.75 ± 1.69	0.11 N.S	
		Female (60)	41.46 ± 1.72		
3	fT (pmol/l)	Male (40)	5.99 ± 0.36	3.32*	
		Female (60)	4.85 ± 0.15		

Hampshire and LWYL were 50.19±3.60 nmol/l, 39.73±2.20 nmol/l, 44.72±1.46 nmol/l, 32.18±2.75 nmol/l and 39.53±4.15 nmol/l respectively.The mean fT_4 concentration observed in LWY, Ghungroo, HDK75, Hampshire and LWYL were 5.13±0.28 pmol/l, 5.02±0.19 pmol/l, 4.68±0.16 pmol/l, 4.31±0.38 pmol/l and 8.37±0.59 pmol/l respectively. Present study reports statistically significant ($p \leq 0.05$) difference within the breeds. The significant deviation in thyroid profile in healthy pigs is in accordance with finding of Petkov (2008), who reported quantitative variations in status of thyroid hormones (TT_3 and TT_4) in various breeds of pigs.

Diet wise thyroid profile: Diet wise deviations in mean concentration of thyroid profile are presented in Table 7. Mean concentration of TT_3 values of pigs having commercial diet and kitchen waste food were 0.75±0.04 nmol/l and 1.19±0.06 nmol/l, respectively. Statistically significant ($p \leq 0.05$) difference was observed within groups.The mean concentration of TT_4 values of pigs having commercial diet and Kitchen waste food were 41.91±1.27 nmol/l and 39.53±4 nmol/l respectively. The mean concentration of fT_4 values of pigs having commercial diet and kitchen waste food were 4.81±0.11 pmol/l and 8.37±0.57 pmol/l respectively. Statistically significant ($p \leq 0.05$) difference was seen within groups. The higher concentration of TT3and fT4 was observed in pigs fed on kitchen waste food diet than pigs offered commercial diet, whereas concentration of TT_4 recorded non-significant($p \leq 0.05$) variation in respect to diet.

In the present investigation, deviation in thyroid profile was noticed with respect to diet. This deviation in thyroid profile is in physiological normal limit with no clinical evidence of thyroid dysfunction (hypo or hyper).

Table 6: Breed wise deviation in thyroid profile in healthy pigs (n=100)

Sr. No	Parameter	Breed	Concentration (Mean ± S.E)	F Value	F Table
1	TT_3 (nmol/l)	LWY (n=13)	0.76 ± 0.08	11.71	0.25
		Ghungroo (n=30)	0.59 ± 0.07		
		HDK75 (n=30)	0.96 ± 0.07		
		Hampshire (n=13)	0.38 ± 0.08		
		LWYL (n=14)	1.19 ± 0.07		
2	TT_4 (nmol/l)	LWY (n=13)	50.19 ± 3.60	4.93	8.57
		Ghungroo (n=30)	39.73 ± 2.20		
		HDK75 (n=30)	44.72 ± 1.46		
		Hampshire (n=13)	32.18 ± 2.75		
		LWYL (n=14)	39.53 ± 4.15		
3	fT_4 (pmol/l)	LWY (n=13)	5.13 ± 0.28	24.79	0.95
		Ghungroo (n=30)	5.02 ± 0.19		
		HDK75 (n=30)	4.68 ± 0.16		
		Hampshire (n=13)	4.31 ± 0.38		
		LWYL (n=14)	8.37 ± 0.59		

Table 7: Diet wise deviation in thyroid profile in healthy pigs (n=100)

Sr. No	Parameter	Type of diet	Concentration (Mean ± S.E)	t cal	t table (p<0.05)
1	TT_3 (nmol/l)	Commercial (n= 86)	0.75 ± 0.04	4.09*	1.98
		Kitchen waste (n= 14)	1.19 ± 0.06		
2	TT_4 (nmol/l)	Commercial (n= 86)	41.91 ± 1.27	0.67 N.S	
		Kitchen waste (n= 14)	39.53 ± 4.00		
3	fT_4(pmol/l)	Commercial (n= 86)	4.81 ± 0.11	9.72*	
		Kitchen waste (n= 14)	8.37 ± 0.57		

Mean should be read column wise for comparison.

It may be considered that the kitchen waste food contains high level of common salt than the commercial food. Therefore, the iodine content in kitchen waste food is high than the commercial diet. The salt requirement for pigs is 0.4-0.5% per kg of diet. Generally, the food which meant for humans consumption was having > 1 to 2 % salt per kg of food. In present study, the pigs which are maintained on kitchen waste food diet showing higher thyroid hormone concentration. This might be due to higher salt concentration in kitchen waste food.

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