

CHAPTER III

IODINE STATUS OF CATTLE AND GOATS IN ZIMBABWEAN COMMUNAL LANDS AS MEASURED BY FREE TETRAIODOTHYRONINE IN SERUM.

AIM

To apply a DPC COAT-A-COUNT (DPC,1997) RIA kit procedure used routinely for human sera to determine the patterns of thyroid hormone levels in cattle and goats raised under natural conditions in Zimbabwe, and to assess factors associated with the levels detected.

MATERIALS AND METHODS

Study design

In the absence of any other knowledge about values of free thyroid hormone levels in cattle and goats in the country, a cross sectional study was preferred. Further, as serological measurements were to be used, a cross-sectional design was considered suitable (Evans *et al.*, 1974). This would be used to establish baseline levels of the iodine status in both cattle and goats as well as identify any risk patterns and principal determinants. The reference populations were the communal cattle and goat populations of Zimbabwe. The sampling unit was therefore the individual cattle and goats in the areas sampled. The populations of interest were the cattle and goat populations in the areas serviced by the dip-tanks. The areas (administrative districts) were selected by formal random sampling into the study from each of the eight

administrative provinces, aiming at sampling from at least one area in each province. Convenience of location of the dip-tank in a selected area, for transportation of samples for preparation and shipping to the Central Veterinary Laboratories in Harare and natural agro-ecological situation were used as secondary criteria for selecting the sampling points. Agro-ecological regions often co-relate to altitudes, such that most places in regions 2 and 1 respectively are on the highveld, 1 200 – 1 700 metres above sea level, and generally receive 900 to 1 500mm of rain annually on average, while summer ambient temperatures seldom exceed 25 -30⁰C. In contrast, regions 5 to 4 are lower lying, 600 – 1000 metres above sea level, are hotter, with peak summer temperatures of 30 – 40⁰C, and tend to receive lower rainfall averaging 400mm per annum. The rest of the country falls in the middle veld, in between these two extremes, generally describing region 3 (APPENDIX III). Local variations of this generalised classification occur often.

However every attempt was made to sample at least two points in each province for representativeness. The study sample size was determined using a single stage sampling calculation based on a mean obtained from a preliminary survey using station animals.

Sampling approach

The sample sizes were determined using the formula $n = (\text{at least}) (sd^2 z^2) / d^2$ described in Daniel (1983). where:

n = sample size estimate

sd = standard deviation (obtained in this case from a small laboratory trial)

z = z value at a confidence level of 95%

d = chosen deviation from the true mean (5% for cattle and 10% for goats)

and a power (1- β) of 90%

Desk determinations showed that in order to estimate levels of FT₄ with a standard deviation of 0.4291 as determined in a sample of station animals, within a 95% confidence level, and a power of 90%, a minimum of 70 individuals needed to be sampled per selected area. Twenty four (24) sites were selected on the basis of natural region and convenience, in consultation with government veterinary officers responsible for each site. This was also in view of accessibility and feasibility of timely transportation of serum, serum separation and transfer to Central Veterinary Laboratory (CVL) in Harare for storage at -20⁰c.

Sampling was conducted between April and August, in 1997 at the diptanks (sites) (Table 3.1).

A systematic sampling technique was used., having determined the number of owners attending the specific dipping days, fair representation of age and sex structures in the animal group present and the total numbers of animals present on the day of sampling, to arrive at the sampling interval based on the predetermined sample size of 35 per site, aiming to sample at least 70 per area (district). As goats are not presented for dipping, volunteer goat owners were enlisted on the sampling day from among those farmers bringing cattle for dipping. Only one flock could be sampled in a day to allow for sample transfer before both cattle and goat samples deteriorated.

Sample treatment and storage

Blood samples from individual animals were collected by veni-puncture into labelled sterile universal bottles, allowed to cool to ambient temperature under shaded areas and then stored in plastic bags on ice to prevent haemolysis. Each bottle was identified by the name of the diptank, date and animal sample number. Further descriptive attributes were entered onto data sheets during sampling for each site. These were names of owners, ages of the animals (by dentition for adults), weight estimates by weigh band, sex, date of last calving for cows, breeds, crown-to- rump lengths, and parity of cows, as well as their lactation status.

In the laboratory, sera were extracted from clotted samples, each aliquoted in triplicates and put into labelled serum vials. These were later to be used for analysis of FT₃, FT₄ and TSH. All samples were stored in a freezer at -20⁰c.

Testing in the laboratory

All samples were tested for FT₄ using a radio-immunoassay (RIA) kit (Diagnostic Products Corporation (DPC, 1997)), Los Angeles, California, USA). The RIA is a solid phase immuno-assay where an ¹²⁵I-labelled analogue, in excess, competes for a fixed time with FT₄ in the test serum for sites on T₄ specific antibody. The specific antibody is immobilised onto the wall of a polypropylene tube. Decanting the supernatant terminates the competition, thereby isolating the antibody-bound fraction of the radio-labelled free T₄. The tube is then read in a Gamma counter. The reading obtained,

converts to a measure of concentration of the free hormone present in the sample, by reading from a calibration curve.

The assay is said to be able to detect down to a level of 0.01 ng/dl with no end-of-run effect observed in assays of up to 250 tubes (DPC, 1997).

The kits contained a set of 7 standards ranging from 0 to a maximum level of 9.6ng/dl. A range of 3 of these standards was included after every 33 samples for quality control. Blank tubes and totals were also included to account for background radioactivity. As this kit is primarily designed for human sera, three human hospital samples representing a low, medium and high concentration of thyroid hormone level, were included at the beginning immediately preceding test samples and at the 97-100th sample as well as at the end, for quality assurance as part of a proficiency testing exercise. Readings were obtained from a computerised gamma counter (LKB WALLAC Multi-Gamma 1261, Finland 1987), following instrument optimization and plotting of a standard curve for each test run, using a set of 7 human serum-based standards as calibrators. Results were accepted only when the laboratory management was satisfied that readings from these sera met established criteria for acceptability. Final readings for FT₄ were given in ng/dl which were the readings analyzed in this study as the dependent variable of interest. Owing to costs, sera were not assayed for FT₃. A total of 561 cattle and 508 goat sera were assayed.

Body weight estimation

Body weights were estimated by use of a graduated weigh band purchased from Milborrows^R in Harare. The weigh band is placed to circumscribe the chest just behind the shoulder blade. The reading so obtained is an estimated weight in kilograms.

Opportunity was also taken to measure the crown to rump length using the length measures on the same tape.

Data management and analysis

All field and laboratory data were entered onto a structured Microsoft Office Excel 1998 database and screened for errors. A preliminary testing of the assumption of homogeneity of variance of the FT₄ data using both boxplots and histograms, indicated a right skewed distribution. Therefore, non-parametric Mann Whitney Test and Kruskal Wallis One-Way Analysis of Variance of Ranks were used for analysis (Daniel, 1983).

Secondary data used as fixed (independent) variables were region, diptank, sex, age and an estimate of body weight. Epiinfo version 6, (1996) and SPSS Version 8 (1998) for Microsoft Windows (1998) were used for descriptive statistics, correlation and other multivariate analyses.

RESULTS

Characteristics of the sample population

A total of 561 cattle and 508 goat sera were subjected to laboratory analysis. These were from 24 sites around the country, identified by the nearest diptank, stratified by age

groups, sex groups as males, females and oxen for cattle, and other factors such as region (agro-ecological) and altitude (Tables 3.1 and 3.2). In cattle, the overall FT₄ mean was 0.51 ng/dl with a standard deviation of 0.260 ng/dl, an overall median value of 0.466 ng/dl (Table 3.1) and a raw data range of 0.089 to 1.900 ng/dl. In goats, the mean and median FT₄ values were 0.93 ± 0.405 ng/dl and 0.863 ng/dl (Table 3.2), respectively, with a raw data range of 0.430 to 1.490 ng/dl (Table 3.2). Therefore both FT₄ mean and median levels for goats were higher than those for cattle.

The population estimates for goats were more normally distributed than for cattle. The age measures for goats were in shorter spans of time, thus rendering farmer recollection more reliable. The differences among the medians in each species, were found to be statistically significant ($p < 0.001$) (Tables 3.1 and 3.2), justifying further examination of the source of these differences.

Table 3.1: Distribution of cattle Serum FT₄ means and medians by district, province, altitude and diptank obtained in a cross-sectional iodine Survey in Zimbabwe, 1997.

District	Altitude	Natural Region	Diptank	Province	Number of samples	Mean FT ₄ (ng/dl) ± standard deviation	p – value for within district	Median FT ₄ (ng/dl)
Bindura	2	2	Guhwa	Mash Central	15	0.699±0.247		0.677
Chikwaka	2	2	Kowoyo	Mash East	24	0.414±0.143	0.0001	0.407*
Chikwaka	2	2	Nyachivi	Mash East	22	0.731±0.238		0.768**
Bikita	2	2	Makotore	Masvingo	34	0.717±0.305		0.653
Karoi	2	2	Kapiri	Mash West	25	0.289±0.110		0.266
Chimanimani	2	2	Nyanyadzi	Manicaland	36	0.554±0.161		0.501
Mazowe	2	2	Mazowe	Mash Central	34	0.473±0.202		0.438
Hwange	3	3	Chisuma	Mat North	19	0.331±0.101	0.0001	0.332*
Hwange	3	3	Mvutu	Mat North	25	0.676±0.287		0.655**
Hwange	3	3	Lupote	Mat North	22	0.544±0.163		0.548**
Mhondoro	3	3	Zimindo-Mamina	Mash West	20	0.514±0.260		0.395
Wedza	2	3	Gadzingo	Mash East	7	0.307±0.095		0.305
Zvishavane	2	3	Mapanzure	Midlands	24	0.661±0.295		0.565
Zaka	2	3	Ndanga	Masvingo	35	0.653±0.261		0.655
Gokwe	3	3	Manyoni	Midlands	15	0.479±0.234	0.1625	0.424*
Gokwe	3	3	Mapfumo	Midlands	14	0.514±0.165		0.487*
Sanyati	3	3	Mudzingwa	Mash West	20	0.441±0.168		0.439
Nyanga	2	3	Resettlement South	Manicaland	34	0.656±0.306		0.625
Lower Guruve	3	4	Dande	Mash Central	39	0.471±0.167		0.464
Muzarabani	3	4	Old Hoya	Mash Central	24	0.657±0.326		0.617
Kezi	3	4	Bada	Mat South	13	0.252±0.058	0.4839	0.251*
Kezi	3	4	Sinti	Mat South	20	0.248±0.101		0.215*
Gwanda	3	5	Gonkwe	Mat south	18	0.298±0.118	0.4917	0.283*
Gwanda	3	5	Mhlatshana	Mat south	20	0.359±0.215		0.296*
			Overall		561	0.514±0.260		0.466

Medians from the same district with different number of superscripts are significantly different

Altitude 2=1200 metres above sea level and higher

Altitude 3= below 1200 metres above sea level

Table 3.2: Distribution of goat serum FT₄ means and medians by district, altitude and diptank obtained in a cross-sectional iodine Survey in Zimbabwe, 1997.

District	Altitude above sea level	Natural Region	Diptank	Number of samples	Serum FT ₄ mean (ng/dl) ± standard deviation	Median serum FT ₄ (ng/dl)
Bindura	2	2	Guhwa	26	2.8±7.59	1.38
Mazowe	2	2	Mazowe	32	1.2±0.34	1.16
Chikwaka	2	2	Nyachivi	18	1.3±0.50	1.22*
Chikwaka	2	2	Chikwaka	15	0.8± 0.22	0.76**
Chimanimani	2	2	Nyanyadzi	36	1.0±0.24	0.91
Bikita	2	2	Makotore	35	1.1±0.44	0.98
Svosve	2	2	Gadzingo	13	1.3±0.43	1.19*
Svosve	2	2	Chepfuma	7	1.5±0.43	1.49**
Hwange	3	3	Lupote	24	1.0±0.28	0.94*
Hwange	3	3	Mvutu	17	1.0±0.27	0.98*
Hwange	3	3	Chisuma	22	0.9±0.31	0.76**
Gokwe	3	3	Dera	11	0.7±0.20	0.77
Gokwe	3	3	Manyoni	26	0.7±0.21	0.72
Lower Guruve	3	4	Dande	35	0.9±0.23	0.90*
Muzarabani	3	4	Kasekete	24	0.6±0.12	0.55**
Nyanga	2	3	Resettlement-south	13	0.8±0.23	0.77
Nyanga	2	3	Mapako	21	0.9±0.28	0.89
Zaka	2	3	Ndanga	35	0.9±0.32	0.80
Zvishavane	2	3	Mapanzure	16	1.5±0.73	1.27
Mhondoro-Ngezi	3	3	Manhizi	10	0.8±0.20	0.74*
Mhondoro-Ngezi	3	3	Zimhindo-mamina	10	0.8±0.33	0.97**
Sanyati	3	3	Mudzingwa	20	0.6±0.24	0.72
Karoi	2	2	Kapiri	16	0.6±0.25	0.60
Kezi	2	4	Bada	14	0.5±0.18	0.49
Kezi	2	4	Maqina	12	0.4±0.14	0.43
Overall				508	0.93±0.405	0.863

altitude 2=1200 metres above sea level and higher

altitude 3= below 1200 metres above sea level

*Medians from the same district with different number of superscripts are significantly different

(p<0.01)

Table 3.3: Distribution by district of Means and Medians of Serum FT₄ for cattle and goats obtained in cross-sectional iodine Survey in Zimbabwe, 1997.

District	Bovine mean FT ₄ (ng/dl) ± standard deviation	Caprine mean FT ₄ (ng/dl) ± standard deviation	Bovine median FT ₄ (ng/dl)	Caprine median FT ₄ (ng/dl)
Bikita	0.7± 0.31	1.1± 0.44	0.65	0.98
Bindura	0.7± 0.25	1.3± 0.53	0.68	1.38
Chikwaka	0.6± 0.191	1.1± 0.45	0.51	0.93
Mazowe	0.5± 0.20	1.2±0.34	0.44	1.16
Wedza	0.3± 0.10	1.3± 0.43	0.31	1.19
Svosve	na	1.5± 0.43	na	1.49
Gokwe	0.5± 0.100	0.7± 0.20	0.46	0.77
Zvishavane	0.7± 0.30	1.5± 0.73	0.57	1.27
Hwange	0.5± 0,184	0.9± 0.29	0.52	0.92
Karoi	0.3± 0.11	0.6± 0.24	0.27	0.60
Mhondoro-Ngezi	0.4± 0.064	0.9± 0.19	0.40	0.97
Nyanga	0.7± 0.31	0.9± 0.26	0.63	0.84
Sanyati	0.4± 0.17	0.8± 0.33	0.44	0.72
Zaka	0.7± 0.26	0.9± 0.32	0.66	0.80
Kezi	0.3± 0.10	0.5± 0.17	0.23	0.43
Muzarabani	0.7± 0.33	0.6± 0.12	0.62	0.55
Lower Guruve	0.5± 0.17	0.9± 0.23	0.46	0.90
Gwanda	0.3± 0.14	na	0.28	na
Overall	0.5± 0.26	0.9± 0.41	0.47	0.86

na= not available

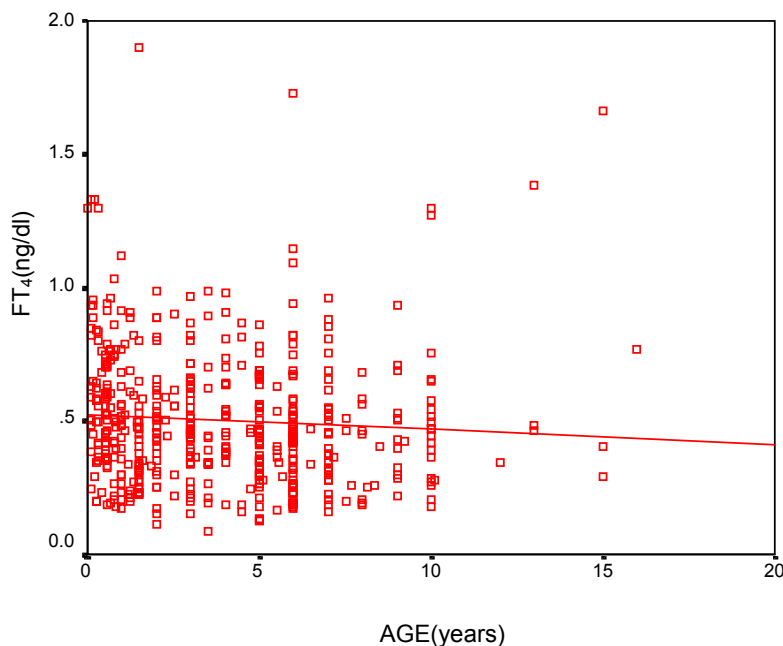
The patterns of the means and medians of FT₄ in the two species were of nearly parallel equivalence among most sites sampled, indicating a general trend (Table 3.3).

The age distribution in cattle was skewed to the right indicating that large numbers of sampled animals were between 0.5 and 4 years (Figure 3.1). In goats, the classification

rendered the sample numbers higher for the 0 to 2 year olds (Figure 3.2). Age in cattle was significantly negatively correlated ($r = -0.152$, $p = 0.01$) to FT_4 , (Fig 3.1). Ages in goats were also significantly negatively correlated ($r = -0.162$, $p = 0.01$) to FT_4 , (Fig 3.2).

Comparatively, goats kids aged 1-3 months and sub-adults aged 4-9 months (Table 3.4) had significantly ($p = 0.001$) higher medians than those older than 9 months. But, the difference between kids and sub-adults was not significant ($p > 0.05$), while the difference between animals less than 9 months (kids, sub-adults) and adults was highly significant ($p = 0.001$).

Poorer age recall for cattle especially in the younger age groups, made this parameter unreliable for comparisons.



$r = -0.152$, $p = 0.01$

Fig 3.1: Scatter plot and a line of best fit of cattle serum FT_4 against age in years in a cross-sectional survey of iodine in Zimbabwe 1997.

Table 3.4: Distribution by age of serum FT₄ means and medians obtained from a cross-sectional survey of goats in Zimbabwe, 1997.

Group	Age in months	Number of animals sampled	Mean serum FT ₄ ± standard deviation	Median serum FT ₄ value	Range of serum FT ₄ value
1	1 – 3 months	53	1.19±0.58	0.96 [*]	0.27-3.56
2	4- 9 months	71	1.02±0.43	0.99 [*]	0.30-2.23
3	> 9 months	250	0.85±0.36	0.79 ^{**}	0.25-2.56

*Medians with the same number of superscript symbol are not significantly different
 Medians with a different number of superscript symbol are significantly different (p < 0.01)

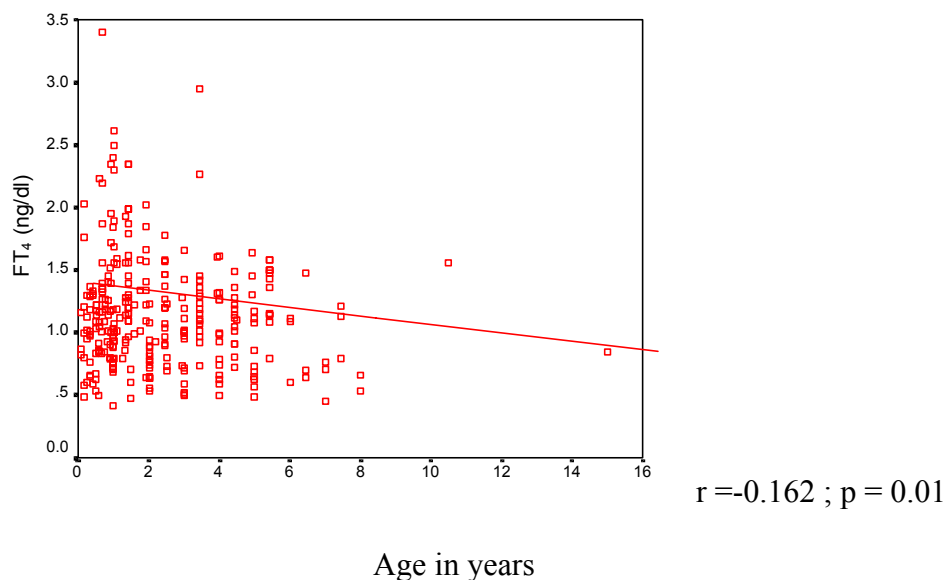
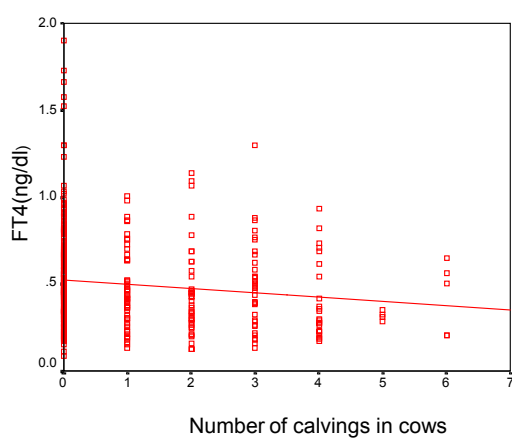


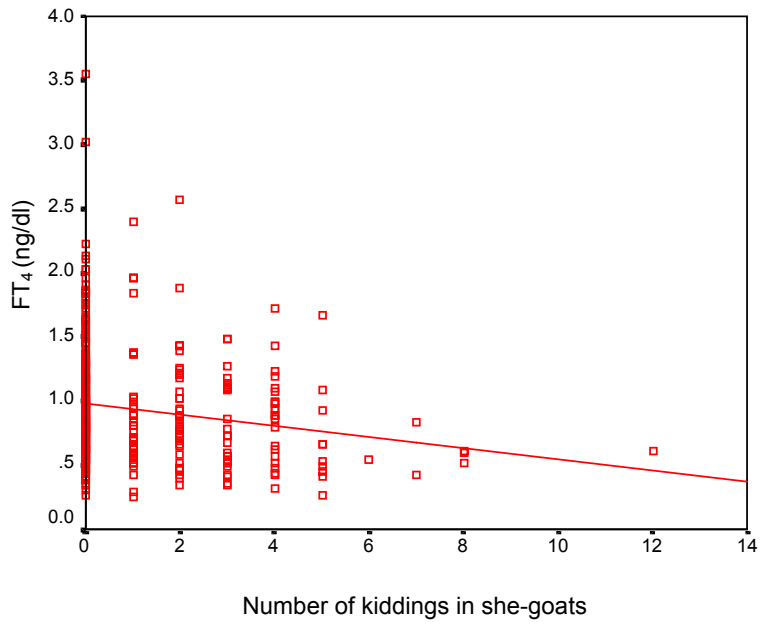
Fig 3.2: Scatter plot and line of best fit of goat serum FT₄ against age in months obtained in a cross-sectional survey of iodine in Zimbabwe 1997.

Figure 3.3 depicts the number of calvings and FT₄. The concentrations of FT₄ decreased as the number of calvings increased ($r = -0.154$, $p = 0.01$). Figure 3.4 showed that goat FT₄ also decreased as the number of kiddings increased ($r = -0.185$, $p = 0.01$). Similar patterns were observed for the relationships between FT₄, age and crown to rump length.



$$r = -0.154; p = 0.01$$

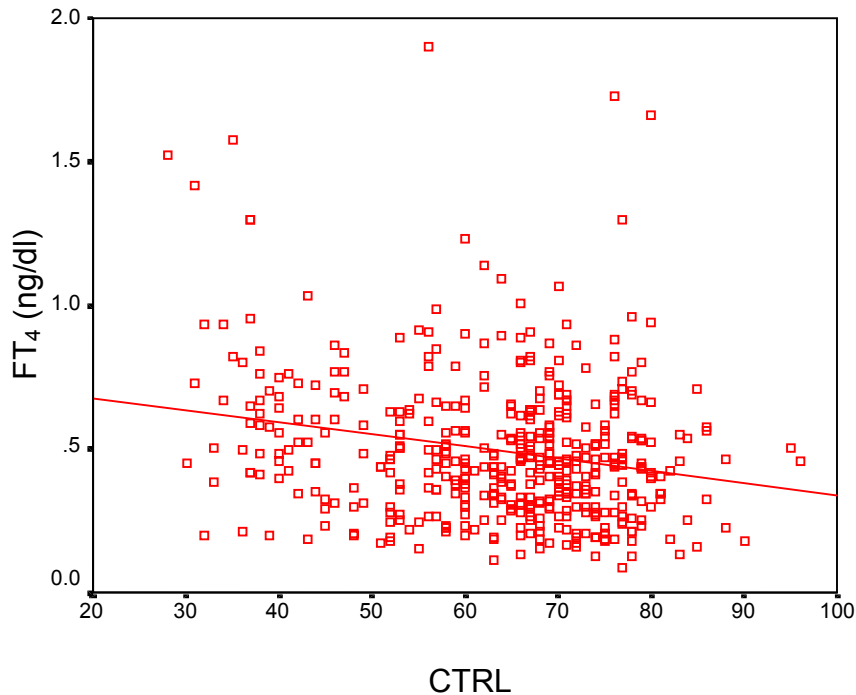
Fig 3.3: Scatter plot and a line of best fit of serum FT₄ against the number of calvings in cows obtained in a cross-sectional survey of iodine in Zimbabwe, 1997.



$r=-0.185$; $p=0.01$

Fig 3.4: Scatter plot and a line of best fit of serum FT₄ against the number of kiddings in goats obtained in a cross-sectional survey of iodine in Zimbabwe, 1997.

FT₄ levels in cattle decreased with increasing crown to rump length (Fig. 3.5).



$$r = -0.217 \quad p = 0.01$$

Fig 3.5 Scatter plot and a line of best fit of goats serum FT₄ levels against the crown-to-rump length (CTRL) obtained in a cross-sectional survey of iodine in Zimbabwe, 1997.

A comparison of lactating and non-lactating cattle at the veterinary field station in Mazowe showed that lactation was significantly ($p=0.001$) associated with lower FT₄ levels (Table 3.5). The general communal cattle sample population however, showed no significant ($p=0.180$) difference in FT₄ levels between males, oxen and females (Table 3.6). This homogeneity was also seen in goats (Table 3.7). There were no significant differences in levels between the sexes in the general population both in cattle and goats.

Table 3.5: Summary of cattle serum FT₄ statistics by lactation status in the Mazowe Station Herd in a cross-sectional survey of iodine in Zimbabwe, 1997.

Lactation status of cows	Number of cows sampled	Mean serum FT ₄ ng/dl± standard deviation	Median serum FT ₄ ng/dl	Range of serum FT ₄ values (ng/dl)
Lactating	10	0.31±0.057	0.286*	0.254 - 0.421
Non-Lactating	24	0.54±0.202	0.502**	0.232 - 0.939

Difference in the number of superscript symbols denotes significant difference in median values (p=0.001).

Table 3.6: Levels of serum FT₄ by sex in the study cattle population obtained in a cross-sectional survey of iodine in Zimbabwe, 1997.

Sex of animals sampled	Number of animals sampled	Mean serum FT ₄ ng/dl± standard deviation	Median serum FT ₄ values (ng/dl)	Range of serum FT ₄ values (ng/dl)
Females	336	0.50 ±0.267	0.445*	0.089-1.900
Entire males	142	0.52±0.234	0.512*	0.113-1.726
Oxen	78	0.55±0.275	0.481*	0.296-1.660
Total sample	556	0.51±0.260	0.465	0.089-1.900

*Medians with the same number of superscript symbols are not significantly different p-value for differences in FT₄ medians among the sexes = 0.180

Table 3.7: Serum level of FT₄ by sex in the study goat population obtained in a cross-sectional survey of iodine in Zimbabwe, 1997.

Sex of animals sampled	Number of animals sampled	Mean serum FT ₄ values (ng/dl) ± standard deviation	Median FT ₄ ng/dl	Range of FT ₄ values (ng/dl)
Female	400	0.942±0.405	0.882*	0.250-3.56
Male	88	0.903±0.336	0.843*	0.270-2.11
Total sample population	508	0.935±0.393	0.867*	0.250-3.56

*Medians with the same number of superscript symbols are not significantly different p-value for differences in FT₄ medians among the sexes = 0.10

Overall, goat FT₄ levels varied significantly ($p < 0.001$) by natural regions. Regions 2 and 3 were significantly ($p = 0.001$) different from Region 4 (Table 3.8). All pairwise comparisons of medians were significantly ($p < 0.05$) different. A similar pattern was evident in cattle, where regions 4 and 5 were lower than regions 2 and 3, with $p < 0.05$. The median FT₄ for natural region 4 was higher than that of 5, $p = 0.01$ (Table 3.9). Significant differences in values between regions in the same district (Table 3.1) were observed in natural region 2 and 3, whereas regions 4 and 5 showed some degree of homogeneity.

Table 3.8: Summary results of pair-wise comparisons of natural region-specific median serum FT₄ levels in the entire study goat population obtained in a cross-sectional survey of iodine in Zimbabwe, 1997.

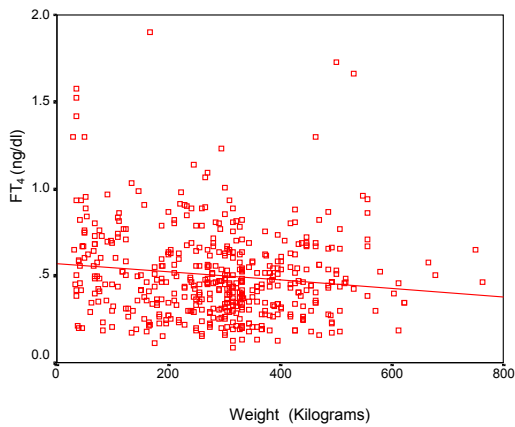
Natural region	Number of animals	Mean FT ₄ levels (ng/dl) ± standard deviation	Median FT ₄ levels ng/dl	Range of serum FT ₄ values (ng/dl)
2	185	1.10± 0.427	0.975*	0.254 - 3.019
3	238	0.92± 0.384	0.851*	0.347 - 3.556
4	85	0.69± 0.277	0.617**	0.259 - 1.537

Medians with different number of superscripts are significantly different (p < 0.01)

Table 3.9: Summary results of pair-wise comparisons of natural region-specific median serum FT₄ levels in the entire study cattle population obtained in a cross-sectional iodine survey in Zimbabwe, 1997.

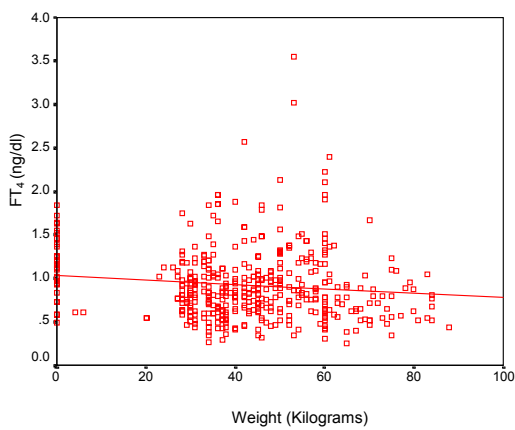
Natural Region	Number of animals sampled	Mean serum FT ₄ levels (ng/dl) ± standard deviation	Median serum FT ₄ levels (ng/dl)	Range of serum FT ₄ levels (ng/dl)
2	192	0.55 ± 0.257	0.500*	0.113 -1.385
3	234	0.55 ± 0.260	0.496*	0.155 -1.900
4	96	0.44 ± 0.253	0.384**	0.089 –1.576
5	39	0.34 ± 0.178	0.314**	0.124 –0.883

Medians with different number of superscripts are significantly different (p < 0.01)



$r = -0.120, p = 0.01$

Fig 3.6: Scatterplot of cattle serum FT₄ levels against body weight obtained in a cross-sectional iodine survey in Zimbabwe, 1997.



$r = -0.104, p = 0.03$

Fig 3.7: Scatterplot of goat serum FT₄ levels against body weight obtained in a cross-sectional iodine survey in Zimbabwe, 1997.

Body weight, estimated by a weigh band, was significantly and positively correlated ($r=0.47, p=0.01$) to the crown-to-rump length (CTRL) (Fig 3.7). The weight distribution curve was nearly normal, in cattle. In goats, this relationship was more definitely linear

up to a weight 30 kg. Above this, there tended to be a clustering of weight estimates into two sub-populations. It may therefore be possible to use the weigh band method with reliability up to a certain level especially that the relationship between thyroxine and body weight may differ between growing and adult animals (Kahl and Bitman, 1983). The correlation of both was however, negative being -0.170 for CTRL and -0.120 (Fig 3.6) for weight estimate with p values for both at 0.01 for cattle. The correlation of weight and FT₄ in goats was also negative and significant, $r=-0.104$; $p=0.03$ (Fig 3.7).

DISCUSSION

FT₄ measurements in serum have been found to provide reliable means for the assessment of thyroid activity in mammals, and have greater sensitivity and specificity than those of total serum T₄ (Nelson and Wilcox, 1996). If the T₄ molecule is universal, and does not vary with species, then the levels detected reflected the status in cattle and goats. Using 3 different kit types, Millar and Alby (1985) confirmed that RIA kits give consistent results for both FT₄ and FT₃ in both sheep and cattle.

FT₄ levels in healthy animals reflect the thyroid status, with a caution that levels might rise with rising body temperatures (Osorio, 1967). Although free thyro-hormones are only 0.03% and 0.3% of protein bound T₄ and T₃ respectively (Nixon et al., 1988), it is these free hormone fractions which are important in eliciting cellular responses (Mendel, 1989). FT₄ is converted to FT₃ and the latter is responsible for cellular reactions in various organs and tissues (Braverman et al., 1970). In deficiency states however, TSH levels only start rising when T₃ and T₄ levels fall by a range of 58 to 59%. It has been shown that arithmetic changes in T₃ and T₄ levels are accompanied by logarithmic changes in TSH levels (Leung et al, 1980; Keffer, 1996). A slight change in free

thyroid hormone may therefore be important when using TSH as the diagnostic measure of choice. Presumably therefore, FT₄ gives a better assessment of the status of thyroid hormone in the blood which otherwise would be provided indirectly by iodine assays. In this study, minute levels of FT₄ were obtained with the human RIA kit. This illustrated that this kit was effective in assaying FT₄ in sera from cattle and goats.

FT₄ levels were found to be variable in cattle and goats at the different sampling regions within a specific district. In Zimbabwe, natural agro-ecological regions (zones) are defined mainly by average annual rainfall, altitude and atmospheric temperature (Ministry of Mines, Environment and Tourism, 1998; APPENDIX III). An attempt to analyse the variations in FT₄ levels using altitude showed that this variable did not significantly influence them. This was an unexpected finding since different altitudes are normally associated with different levels of agro-potential regions. Altitude is known to influence the iodine levels both in animals and the environment (McDowell *et al.*, 1984b). This finding can be hypothesised to be due to altitude being either too “general a classifier” or be confounded by other factors like rainfall, soil characteristics, animal breeds and grazing patterns.

Relatively lower levels of FT₄ were found in regions 4 and 5 than in regions 2 and 3 in both cattle and goats. This might explain the apparently faster growth rates indicated by weight gains in cattle and goats in regions 4 and 5. Presumably this is due to a more rapid conversion of FT₄ into FT₃ in both cattle and goats in regions 4 and 5. This phenomenon may not occur to the same extent in regions 2 and 3, resulting in apparently higher serum FT₄ levels which is not the metabolically active form of thyroid hormone (Kahl and Bitman, 1984). The relationship of thyroid hormone and growth is two-pronged. According to Rumsey *et al.* (1990), increased metabolism of T₄ is part of a

mechanism by which bovine growth hormone regulates growth and tissue deposition. Under euthyroidic conditions, levels of T_4 fall as they convert to T_3 while effecting growth of muscle, bone and other tissues mediated by the growth hormone (Rumsey et al., 1990). T_3 is considered to be key in both lipogenesis (Oppenheimer et al, 1987) and protein deposition (Brown et al., 1983; Rumsey et al., 1990). It is reasonable to expect an equilibrium as long as iodine levels are maintained. Growth might also be reflected in weight gain as hormone levels are kept low as a result of goitrogens or in iodine deficiency states as a result of low energy metabolism (Rumsey et al, 1985b; 1988) through inhibition of the deiodination of T_4 . Higher retention of water and salts reflecting effects of thyroid status on the adrenals (Robertson et al, 1957; Underwood, 1971) and presumably, reduced fat metabolism resulting in higher fat deposits might be expected in such cases.

Low metabolism as a result of extreme hypothyroidism in new-borns and the young could also lead to stunting as a result of failure in protein building, a parallel of cretinism in humans, while the adults may suffer from myxedema (Merck Veterinary manual, 1979). It is common knowledge in Zimbabwe that cattle on the highlands, mostly regions 1 and 2 are smaller in stature and they grow and mature more slowly than those in regions 4 and 5. In the field, general observations were also made during the present study, on the slow growth rate of animals on the high veld in contrast to that observed in the medium to lower altitude areas such as Gwanda, an area typically renowned for beef production. Relative to regions 4 and 5 therefore, cattle in the highveld might be displaying a hypothyroidic effect leading to lower weight gains. This could be exacerbated where environmental iodine levels are low. Anecdotal reports by animal production advisors and practising veterinarians have often attributed low performance

on the highveld to in-breeding, breed type and the competition between cropping and pasture lands.

In this study, higher serum levels of FT₄ in both cattle and goats were found in the wetter, higher regions than in the lowveld. It can therefore be surmised that thyroid activity is relatively lower due to metabolic unavailability of the active hormone, where the hormone accumulates as FT₄ rather than its active (FT₃) form. Visibly goitrous lambs were seen during this study in Chikwaka, Svosve and Wedza which are on the highveld, reflecting a relative deficiency status in natural region 2. It would also appear that high thyroxin (FT₄) activity keep its levels in cattle low in the otherwise iodine-abundant low veld environment as described in chapter IV. When excessive iodide is fed, thyrotoxicity results, leading to sub-normal T₄ and T₃ production (Leung et al, 1976) while, TSH levels rise. TSH levels may then also change in direct relationship with T₄ levels in pituitary dependent abnormalities (Stockigt, 1996). Iodine deficiency might therefore lead to such a situation either due to primary or secondary reasons. Interpretation of hormone levels therefore needs caution and it may be necessary to simultaneously measure FT₃ and TSH levels in the same subjects.

The value of TSH might however vary given the extent and duration of the fluctuation of thyroid hormone. Natural regions, generally follow vegetation patterns which may define nutritional differences. Some obvious differences are the sweetveld and the mopani woodlands of the lower lying regions 4 and 5 as opposed to the acacias and the msasa woodlands of natural regions 2 and parts of 3. Secondly, iodine which is washed down or leached from higher lying regions 2 and 3 is likely to accumulate in the lower lying regions 4 and 5.

It was observed in this study that age was negatively correlated with FT₄ levels in cattle. However, it would be necessary to verify whether this observation signifies an increased or decreased thyroid hormone activity. Ridgway (1996) stated that primary thyroid failure affected 10-15% of populations in the USA, was more common in females, and increased with age. This might imply that the decline was related to primary iodine deficiency or increased pressure for its requirement. It should however be noted that in the absence of accurate records of age, this parameter might be compounded by estimation, except in the very young. Body weight, a proxy for age in this study gave a more negative correlation. However, this relationship with weight may reflect effects of higher FT₄ hormone transformation into FT₃. This would translate into higher protein tissue deposition or lower tissue breakdown and hence higher weight gains. This could also explain a principle utilized by beef producers in achieving weight gains in beef animals through the use of goitrogenics such as thiouracil (Stasilli et al, 1960; Escobar and Morreale, 1961). While in suckling animals, high FT₄ levels could be a result of high iodide from milk feeding, particularly from colostrum, the declining levels could relate to low iodide levels, thyrotoxicity or increased hormone metabolism as it transforms into FT₃.

It was of interest to confirm the usefulness of CTRL as a surrogate of weight determination. It would seem however that this means of estimation of weight in goats may vary with age, wherein the relationship occurs clustered into two groups. This study has illustrated that CTRL is a measurement that can effectively substitute for weight. Its convenience in a communal setting is that it can be estimated by a straight solid object instead of a graduated tape measure. In situations where iodide imbalances

are suspected, a standard curve of CTRL against FT₄ could be used to read off expected results.

Another factor which could exacerbate the age effect is the further confirmation in the Mazowe dairy herd group that lactating cows could be expected to have significantly lower levels of iodine. This could therefore lead to reduction in FT₄ levels in euthyroid populations especially where hypothyroidism might exist (Sorensen 1962). That this effect was not demonstrated in the general communal cattle and goat populations could mean that lactation naturally does not deplete iodide reserves under these conditions. This is perhaps consistent with the fact that milk yields are uniformly low and therefore present low metabolic pressure, or that at the time of sampling, not many dams were at the height of lactation. The general observation and responses by farmers indicated that daily milk yields are only 1-2 litres per cow at the time of this study. Programs to increase milk production in communal areas might therefore need to be accompanied by mineral supplementation in this case, iodine.

Sex on its own, also did not appear to be important for hormone levels in both cattle and goats implying that in communal settings sex differences, including the influence of work on oxen are not an issue.

Calves in communal areas tend to suckle for longer than normally recommended for commercial production. This has the potential of introducing an apparent deficiency in iodine and hence differences in FT₄ levels between male and female cattle. Visible and palpable goitre was frequently “diagnosed” in goat kids and lambs at Chikwaka, Svosve and Wedza. This observation was not corroborated by the low levels of FT₄ recorded

in these young kids. Nevertheless, this could be either due to a compensatory effect in the goitrous animals, especially as other studies have failed to substantiate reduced activity even in the presence of demonstrable goitre (McCoy et al., 1997), unless under those conditions, it is the inactive FT₄ form that features.

CONCLUSION

In conclusion, this study produced some data on serum levels of FT₄ which can be used at national level. Several factors influenced these medians, notably the agro-ecological regions. The significant variations associated with these natural regions indicate a need to map up the serum levels of FT₄ by regional medians. Results in Table 3.1 showed that there was greater heterogeneity in the distributions of serum levels of FT₄ in highveld than those recorded in the lowveld. Discounting effects from other factors, the higher weight for age observed in region 4 and 5, particularly in Matebeleland South (Gwanda) could partly be explained by the lower thyroxine hormone levels, possibly due to higher conversion of FT₄ to the active FT₃ in an environment of high forage iodide content as recorded in chapter IV. This is likely attributable to weight gain by protein tissue development. FT₄ levels seemed to decline in situations where performance was enhanced by such factors as increased age, high lactation, and feeding antithyretics.

Due to differences in geographical factors, breed and function, it was not possible to make meaningful comparison with the only other data sets identified for cattle in a North American study (Refetoff, 1970). No data could be identified for comparisons in goats.