

**APPROACHES FOR EPIDEMIOLOGICAL INSIGHTS INTO THE  
IODINE NUTRITION OF RUMINANT LIVESTOCK UNDER  
NATURAL CONDITIONS IN**

**ZIMBABWE**

**By**

**UNESU HILDA FORTUNE USHEWOKUNZE-OBATOLU**

**A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of  
Philosophy**

**Department of Clinical Veterinary Studies  
Faculty of Veterinary Science  
University of Zimbabwe**

**December, 2004**



## **DEDICATION**

**I dedicate this thesis to all those who value the satisfaction that comes with perseverance. In particular my late father, S.M.M. Ushewokunze whose motto to family was “if others can, why can’t I?” and my late mother who was resolute that a lot of solutions are a result of common sense.**

**I also dedicate this thesis to my husband Oluseye and my only daughter Fadeke, who stood by me in demanding times when I had to stay up late and often, away from home both physically and in mind, in order to make my dream of self-actualisation a reality.**

## ABSTRACT

This thesis is based on work founded on an hypothesis that Zimbabwe is endemic for hypothyroidism as previously indicated in human studies, therefore a case for hypothyroidism for livestock was presumed.

The studies conducted for this thesis sought to establish population diagnostic systems for thyroid status, hence iodine status, that could be used in cattle and goats while establishing some reference values and epidemiological factors that could be used in herd health decision-making.

The approaches used for the epidemiological studies included a population cross-sectional study using cattle and goat sera, testing of pasture materials for iodide levels, a station supplementation experimental study and a farmer participatory field trial testing an iodised feed supplement. The diagnostic methods tested included radiomunoassay (RIA) for thyroid hormones, the iodide ion selective electrode (ISE) method for feed materials and the enzyme linked immunosorbent assay (ELISA) for serum thyroid stimulating hormone (TSH).

The RIA was able to detect serum free triiodothyronine and free tetraiodothyronine in cattle and goats. The results are presented confirming the effect of factors such as animal age, body weight, sex, lactation status and natural agro-ecological region in cattle and goats. The range for cattle was 0.089 to 1.900ng/dl with a median of 0.46ng/dl; while for goats the range and median were 0.430 to 1.49 ng/dl and

0.933ng/dl, respectively. An attempt to validate the RIA findings in cattle against levels of thyrotropin did not succeed because the ELISA method attempted suffered from cross-reaction with leutenising hormone (LH) and follicle stimulating hormone (FSH).

Despite that observations of goitre in goat kids were made in an area previously recorded as highly endemic for human hypothyroidism, a field trial involving iodine supplementation to dairy cattle in the same area could not substantiate hypothyroidism in lactating animals. On the contrary, the iodine containing supplement appeared to depress milk yield. Similarly, a controlled station experiment with weaner calves could not indicate a performance advantage resulting from iodine supplementation.

The studies have therefore established the RIA and the ISE as methods which can be used to study the iodine status of cattle and goats and some data to assist further investigations into supplementation regimes aimed at improving productivity in cattle and other livestock.

## ACKNOWLEDGEMENTS

The invaluable assistance in the tedious literature search by the librarian Mr C Madziwa is gratefully acknowledged, so is the support to him by the late Mrs Grace Makoni.

For laboratory analysis, I am most thankful to Mrs Anne Bernie and Mrs Esi Chinyanga for their most valuable technical support on the RIA assays. I gratefully acknowledge the leadership of Dr Suman Mahan on the ELISA assays which were empirically generated with his guidance and the excellent support of Ms Shalt Semu. I thank Mrs Lydia Mabikacheche for her untiring work in the evaluation of the ELISA test and Mrs Ethel Nyagani for valuable advice on the chemical calculations for the supplementation study at Mazowe. Dr I. Mpfu and Mr Govere both of University of Zimbabwe (UZ) Animal Science were instrumental in UZ accepting to host and guide the ion selective electrode (ISE) work on feeds at the University of Zimbabwe. For that, I am most grateful.

The arduous task of statistical analysis would not have been entirely accomplishable without the unwavering back-stopping support of the biometrician, Mrs Theresa Munyombwe-Choga. My invaluable personal assistants for many years, Mrs Erica Zvaita and the late Mr O Chinyandura, worked tirelessly on the innumerable preliminary drafts of proposals, publications, reports and finally this thesis. I thank them for being so loyal in support of my research and thesis.

I thank Dr W.N. Madzima and his team of field Veterinary staff for opening the way to let me work with them and farmers at all the points that were used in this study. In particular, I am thankful for the loyal and honest support of Mr Farai Tinarwo, for being my energetic right hand in working with the farmers of the Chikwaka small-holder dairy scheme and rallying them to understand the basis of the work I was doing with them. Similarly, I am indebted to Mr Hilton Majonga and Dr O Kakono for looking after experimental animals at the Mazowe

Veterinary Field Station and ensuring the safekeeping and transfer of samples to the laboratory in Harare regularly during the course of that study.

My utmost thanks go to my supervisors at various stages. I am most grateful to Dr A Majok for his support and suggestions at the initial stages of the project conception and design. Others whose backstopping was crucial were Prof A Brand, Prof Van Leengoed, Dr Hilda T. Marima-Matarira; and for giving me the last push when hope almost failed me, Professors A Bobade and M Obwolo. External oversight support by Professor Karl Zessin and Dr Moses Kyule of the Free University of Berlin, Germany is acknowledged with thanks.

This work would not have been possible without the funding from the European Union arrangement for local research through the University of Zimbabwe. I remain grateful to the Faculty of Veterinary Science for recognizing the importance of supporting development-oriented veterinary research by public sector researchers.

Finally, I wish to thank the Ministry of Lands, Agriculture and Rural Resettlement, for recognizing the importance of permitting capacity enhancement in senior research management by allowing these studies to be carried out under their auspices

## CONTENTS

<b><u>CHAPTER</u></b>	<b><u>PAGE</u></b>
DECLARATION	ii
DEDICATION	iii
ABSTRACT	iv-v
ACKNOWLEDGEMENTS	vi-vii
INTRODUCTION	1-5
GENERAL LITERATURE REVIEW	6-34
IODINE STATUS OF CATTLE AND GOATS IN ZIMBABWEAN COMMUNAL LANDS AS MEASURED BY FREE TETRAIODOTHYRONINE IN SERUM	35-61
DETERMINATION OF IODIDE ION LEVELS IN PASTURES USING THE IODIDE- ION SELECTIVE ELECTRODE (ISE) METHOD	62-73
EXPERIMENTAL STUDY ON THE EFFECT OF IODINE SUPPLEMENTATION ON THE PERFORMANCE OF YOUNG CATTLE AT THE MAZOWE FIELD STATION	74-91
EFFECT OF IODINE SUPPLEMENTATION ON THE MILK YIELD OF COWS IN A COMMUNAL DAIRY SCHEME	92-103
AN ATTEMPT TO DEVELOP AN ELISA TECHNIQUE FOR THE DETERMINATION OF THYROID STIMULATING HORMONE IN CATTLE	104 – 123
GENERAL DISCUSSION AND CONCLUSION	124-132

<b><u>CHAPTER</u></b>	<b><u>PAGE</u></b>
<b>MATERIALS AND PROCEDURES FOR THE ELISA PROCEDURES</b>	<b>133-164</b>
<b>IODATE DOSAGE CALCULATION</b>	<b>165-166</b>
<b>NATURAL REGIONS MAP OF ZIMBABWE</b>	<b>167</b>

## 2. LIST OF FIGURES

<b>Fig 2.1:</b>	<b>Biochemical structure of <math>T_3</math> and <math>T_4</math> .</b>	<b>12</b>
<b>Fig 3.1:</b>	<b>Scatter plot and a line of best fit of cattle serum <math>FT_4</math> against age in years in a cross-sectional survey of iodine in Zimbabwe, 1997.</b>	<b>45</b>
<b>Fig 3.2:</b>	<b>Scatter plot and line of best fit of goat serum <math>FT_4</math> against age in years obtained in a cross-sectional survey of iodine in Zimbabwe, 1997.</b>	<b>46</b>
<b>Fig 3.3:</b>	<b>Scatter plot and a line of best fit of serum <math>FT_4</math> against the number of calvings in cows obtained in a cross-sectional survey of iodine in Zimbabwe, 1997.</b>	<b>47</b>
<b>Fig 3.4:</b>	<b>Scatter plot and a line of best fit of serum <math>FT_4</math> against the number of kiddings in goats obtained in a cross-sectional survey of iodine in Zimbabwe, 1997.</b>	<b>48</b>
<b>Fig 3.5:</b>	<b>Scatter plot and a line of best fit of goats serum <math>FT_4</math> levels against the crown-to-rump length (CTRL) obtained in a cross-sectional survey of iodine in Zimbabwe, 1997.</b>	<b>49</b>
<b>Fig 3.6:</b>	<b>Scatterplot of cattle serum <math>FT_4</math> levels against body weight obtained in a cross-sectional iodine survey in Zimbabwe, 1997.</b>	<b>53</b>
<b>Fig 3.7:</b>	<b>Scatterplot of goat serum <math>FT_4</math> levels against body weight obtained in a cross-sectional iodine survey in Zimbabwe, 1997.</b>	<b>53</b>
<b>Fig 5.1.</b>	<b>Trends of weekly Serum <math>FT_4</math> means during the Mazowe Station Iodide supplementation study, 1998.</b>	<b>80</b>
<b>Fig 5.2:</b>	<b>Co-relationship between serum <math>FT_3</math> and <math>FT_4</math> levels in the experimental group during the Mazowe Station iodide supplementation study, 1998.</b>	<b>81</b>

## LIST OF FIGURES

<b>Fig 5.3:</b>	<b>Co-relationship between serum FT<sub>3</sub> and FT<sub>4</sub> levels in the control group during the Mazowe Station iodide supplementation study, 1998.</b>	<b>82</b>
<b>Fig 5.4 a:</b>	<b>Patterns of weights and FT<sub>4</sub> in control calves group during the Mazowe Station Iodide supplementation study, 1998.</b>	<b>83</b>
<b>Fig. 5.4b:</b>	<b>Patterns of weights and FT<sub>4</sub> in experimental calves group during the Mazowe Station Iodide supplementation study, 1998.</b>	<b>83</b>
<b>Fig 5.5:</b>	<b>Scatter plot of FT<sub>4</sub> levels against age in the experimental group during the Mazowe station iodide supplementation study, 1998.</b>	<b>84</b>
<b>Fig 5.6:</b>	<b>Scatter plot of FT<sub>4</sub> levels against age in the control group during the Mazowe station iodide supplementation study, 1998.</b>	<b>85</b>
<b>Fig. 6.1:</b>	<b>Average monthly milk yields of field trial cows in the Chikwaka communal area. Iodine supplementation field trial, 2000-2001.</b>	<b>99</b>

## LIST OF TABLES

<b>Table 3.1:</b>	<b>Distribution of cattle serum FT<sub>4</sub> means and medians by district, province, altitude and diptank obtained in a cross-sectional iodine Survey in Zimbabwe, 1997.</b>	<b>42</b>
<b>Table 3.2:</b>	<b>Distribution of goat serum FT<sub>4</sub> means and medians by district, altitude and diptank obtained in a cross-sectional iodine Survey in Zimbabwe, 1997.</b>	<b>43</b>
<b>Table 3.3:</b>	<b>Distribution by district of means and medians of Serum FT<sub>4</sub> for cattle and goats obtained in cross-sectional iodine Survey in Zimbabwe, 1997.</b>	<b>44</b>
<b>Table 3.4:</b>	<b>Distribution by age of serum FT<sub>4</sub> means and medians obtained from a cross-sectional survey of goats in Zimbabwe, 1997.</b>	<b>46</b>
<b>Table 3.5:</b>	<b>Summary of cattle serum FT<sub>4</sub> statistics by lactation status in the Mazowe station herd in a cross-sectional survey of iodine in Zimbabwe, 1997.</b>	<b>50</b>
<b>Table 3.6:</b>	<b>Levels of serum FT<sub>4</sub> by sex in the entire study cattle population obtained in a cross-sectional survey of iodine in Zimbabwe, 1997.</b>	<b>50</b>
<b>Table 3.7:</b>	<b>Serum level of FT<sub>4</sub> by sex in the entire study goat population obtained in a cross-sectional survey of iodine in Zimbabwe, 1997.</b>	<b>51</b>
<b>Table 3.8:</b>	<b>Summary results of pairwise comparisons of natural region-specific median serum FT<sub>4</sub> levels in the entire study goat population obtained in a cross-sectional survey of iodine in Zimbabwe, 1997.</b>	<b>52</b>

## LIST OF TABLES

<b>Table 3.9:</b>	<b>Summary results of pairwise comparisons of natural region-specific median serum FT<sub>4</sub> levels in the entire study cattle population obtained in a cross-sectional iodine survey in Zimbabwe, 1997.</b>	<b>52</b>
<b>Table 4.1:</b>	<b>Distribution of forage iodide levels by type of forage grazed by cattle in various communal grazing areas in Zimbabwe, 1997.</b>	<b>66</b>
<b>Table 4.2:</b>	<b>Iodide levels in extracts of forages of cattle in µg/kg by natural agro-ecological region in Zimbabwe, 1997.</b>	<b>67</b>
<b>Table 4.3:</b>	<b>Distribution of iodide levels in forage types by natural regions in communal grazing areas in Zimbabwe, 1997.</b>	<b>68</b>
<b>Table 5.1:</b>	<b>Mazowe Station Iodide supplementation experiment: Serum FT<sub>4</sub> means and medians (ng/dl) in treatment and control groups, 1998.</b>	<b>79</b>
<b>Table 6.1:</b>	<b>Average monthly milk yield per cow by treatment. Chikwaka dairy iodine supplementation field trial, 2000-2001.</b>	<b>96</b>
<b>Table 6.2:</b>	<b>Average monthly milk yield per cow by season and treatment. Chikwaka dairy iodine supplementation field trial, 2000-2001.</b>	<b>98</b>
<b>Table 7.1:</b>	<b>Evaluation of an ELISA for bovine TSH by the indirect reaction format, 2000.</b>	<b>112</b>
<b>Table 7.2:</b>	<b>Evaluation of the indirect TSH ELISA test controlling for the effects of FSH and LH by adsorption, 2000</b>	<b>113</b>
<b>Table 7.3:</b>	<b>Indirect ELISA for evaluating pre- and post- immunization sera against TSH, FSH and LH antigens, 2000.</b>	<b>114</b>
<b>Table 7.4:</b>	<b>Indirect ELISA for examining the cross-reactions of Pre- and Post- immunization sera following pre-adsorption with FSH, LH and TSH on coated plates, 2000.</b>	<b>115</b>

## LIST OF TABLES

<b>Table 7.5:</b>	<b>Sandwich ELISA for TSH antibody levels in goat sera, checking the effect of cross-reaction to FSH and LH, 2000.</b>	<b>116</b>
<b>Table 7.6:</b>	<b>OD readings of a checkerboard Titration of bovine TSH and pre-inoculation goat sera: Determination of dilution levels of test sera and TSH levels using sandwich ELISA, 2000.</b>	<b>117</b>
<b>Table 7.7:</b>	<b>OD readings of a checkerboard titration of bovine TSH and foetal calf serum to check for non-specific reactions using fetal calf serum, 2000.</b>	<b>118</b>
<b>Table 7.8:</b>	<b>OD readings of a checkerboard titration of antibovine TSH against normal bovine serum (NBS).</b>	<b>119</b>

#### 4. LIST OF ACRONYMS AND ABBREVIATIONS

<b><u>ACRONYM</u></b>	<b><u>EXPANDED FORM</u></b>
<b>ABTS</b>	<b>2,2'-azinobis-bis(3-ethylbenz-thiazoline-6-sulfonic acid) diammonium salt</b>
<b>BEI</b>	<b>Butanol extractable iodine</b>
<b>ANCOVA</b>	<b>Analysis of covariance</b>
<b>ARC</b>	<b>Agricultural Research Council</b>
<b>CTRL</b>	<b>Crown-to-rump length</b>
<b>CV</b>	<b>Coefficient of variation</b>
<b>dc</b>	<b>direct current</b>
<b>DIT</b>	<b>Diiodotyrosine</b>
<b>DPC</b>	<b>Diagnostic Products Corporation</b>
<b>dl</b>	<b>Deci-litre</b>
<b>EDDI</b>	<b>Ethylene diamine-dihydroiodide</b>
<b>ELISA</b>	<b>Enzyme linked immunosorbent assay</b>
<b>FA</b>	<b>Freund's antigen</b>
<b>FCA</b>	<b>Freund's complete antigen</b>
<b>Fig.</b>	<b>Figure</b>
<b>FSH</b>	<b>Follicle stimulating hormone</b>
<b>FT<sub>3</sub></b>	<b>Free triiodothyronine</b>
<b>FT<sub>4</sub></b>	<b>Free thyroxine (tetraiodothyronine)</b>
<b>GC</b>	<b>Gas chromatography</b>
<b>HRP</b>	<b>Horse radish peroxidase</b>
<b>IgG</b>	<b>Immnglobulin gamma</b>
<b>ISE</b>	<b>Ion selective electrode</b>
<b>kg</b>	<b>Kilogram</b>

**ACRONYM****EXPANDED FORM**

<b>KPL</b>	<b>Kirkgaard Perry Laboratory</b>
<b>KOH</b>	<b>Potassium hydroxide</b>
<b>LC</b>	<b>Liquid chromatography</b>
<b>LH</b>	<b>Leutenising hormone</b>
<b>MS</b>	<b>Microsoft</b>
<b>mg</b>	<b>milligram</b>
<b>ml</b>	<b>millilitre</b>
<b>mv</b>	<b>millivolt</b>
<b>µg</b>	<b>microgram</b>
<b>µl</b>	<b>microlitre</b>
<b>ng</b>	<b>nanogram</b>
<b>NHPP</b>	<b>National hormone and pituitary programme</b>
<b>NRC</b>	<b>National Research Council</b>
<b>OD</b>	<b>Optical density</b>
<b>p</b>	<b>probability of getting as extreme a value given the null hypothesis is true</b>
<b>PBI</b>	<b>Protein-bound iodine</b>
<b>ppm</b>	<b>Parts per million</b>
<b>ppb</b>	<b>Parts per billion</b>
<b>PBS</b>	<b>Phosphate buffered saline</b>
<b>r</b>	<b>correlation coefficient</b>
<b>RIA</b>	<b>Radio-immuno assay</b>

**ACRONYM****EXPANDED FORM**

<b>T<sub>3</sub></b>	<b>Triiodothyronine</b>
<b>T<sub>4</sub></b>	<b>Thyroxine (tetraiodothyronine)</b>
<b>TBG</b>	<b>Thyroid binding globulin</b>
<b>TRH</b>	<b>Thyroid releasing hormone</b>
<b>TSH</b>	<b>Thyroid stimulating hormone or Thyrotropin</b>
<b>x</b>	<b>Horizontal axis</b>
<b>y</b>	<b>Vertical axis</b>