CHAPTER IV

DETERMINATION OF IODIDE ION LEVELS IN PASTURE MATERIALS USING THE IODIDE-ION SELECTIVE ELECTRODE METHOD.

AIM

To determine the pattern of iodine levels in field pasture materials, and to assess the value of the Iodide-ion Selective Electrode (ISE) technique as a method for routine laboratory assay for iodide levels.

MATERIALS AND METHODS

The study areas, target livestock populations, sampling and other design details have been described in Chapter III.

Collection of forage samples

At each point where cattle blood samples were collected between April and July (dry season) in 1997 during the cross-sectional iodine survey (described in Chapter III), farmers and livestock technicians were asked to indicate which pasture materials the cattle tended to forage in the field. A total of 124 samples comprising grasses, tree leaves, and fruit pods were collected from 24 points following a transect made in the grazing area. An amount of at least 50 grams of each forage material indicated by the farmers and stockmen was taken. The samples were taken by cutting the forage

material 5 centimetres above ground, using a stainless steel scissors. This procedure generally followed an outline of collection methods by Fick et al., (1979). Each sample was separately stored in plastic bags labelled with the farmer name, date of collection, area and name of nearest diptank, as well as local or biological name of the plant.

Preparation of samples

In the laboratory, each sample was oven dried at 60^0C for 6 hours to remove all moisture. The dried samples were individually ground by pestle and mortar and stored in labelled plastic bottles. The samples were processed following a digestion method described by Miles (1978) as follows:

Ten grams (gm) of each sample were weighed into a nickel crucible. Blanks of 2gm soluble starch were carried in parallel through the entire procedure. Ten percent potassium hydroxide (KOH) in ethanol was added and mixed thoroughly before the sample was dried and charred on a hot plate. This was then followed by ashing in a muffle furnace for an hour at 550°C. Thirty millilitres of distilled water were added to the ash and mixed with a glass rod. With the crucible covered, the mixture was boiled for 10 minutes. The ash extract was filtered into a 100ml volumetric flask and diluted to volume.

Iodide measurement

A 50ml sample was pippetted into a 100ml beaker containing a stirring bar. The beaker was placed on a foam pad on top of a magnetic stirrer which was adjusted to the appearance of a vortex. Electrodes were lowered into the test solution. The electrometer function switch was turned to the "mv" position, and using a timing period of 10 minutes, E_1, the potential to the nearest 0.1 mv was recorded. A 5ml volume of a potassium iodide solution of known concentration was added. The concentration of the spiking solution was approximately 10 times that of the sample. Five minutes afterwards, the potential was recorded as E_2. The electrometer was then returned to standby mode. Electrodes were then removed from the test solution, rinsed thrice with distilled water, and wiped gently in preparation for the next test sample.

The electro-potentiometric readings obtained were converted into concentration of iodide in the forage sample in µg/kg using Nernst equation (Miles, 1978) as follows:

$$C = \frac{\{C_a - V_a \ \text{antilog}[(E_2 - E_1)/0.059]\}}{\{V + V_a - V\text{antilog}[(E_2 - E_1)/0.059]\}}$$

Where:

C and C_a are concentrations of test solution and spiked solution, respectively in µg/kg.

V and V_a are volumes of test solution and spiking solution of known concentration respectively.

E_1 and E_2 are potentiometer measurements.

0.059 is a constant

The final readings of concentration were obtained by subtracting the readings of the blank samples.

All the laboratory and field data were managed using Microsoft Office Excel (2000) database and analysed with SPSS version 10 for Windows (2000), using tne one way ANOVA statistical model (Daniel, 1983).

RESULTS

The results of the calculated concentrations obtained showed wide variations (Tables 4.1, 4.2 and 4.3), with grasses and leaves seeming to hold much higher levels than fruits (Table 4.1) particularly in regions 2a and 4 (Table 4.2). Therefore, the data were normalized by square root transformation to stabilise the variances before being analysed. No significant ($p>0.05$) differences were detected among the various forage types within each region. Also, when grasses, leaves and fruits were compared separately between regions, no significant ($p>0.10$) differences were observed.

Table 4.1: Distribution of forage iodide levels by type of forage grazed by cattle in various communal grazing areas in Zimbabwe, 1997.

Forage class	Sample size	Mean forage iodide levels(μg/kg) ± standard deviation	Median forage iodide values (μg/kg)	Square root transformed (geometric) iodide means (μg/kg) ± standard deviation	Square root transformed geometric iodide medians (μg/kg)
Grasses	51	18.1 ± 93.65	1.31	2.1 ± 3.73*	1.15
Leaves	39	18.0 ± 49.30	1.16	2.6 ± 3.36*	1.07
Fruits	9	11.7 ± 27.92	1.09	2.2 ± 2.80*	1.04
TOTAL	99	10.0 ± 66.21	1.21	2.5 ± 3.47*	1.00

*Forage type geometric mean with same number of superscripts are not significantly different ($p>0.05$).

Table 4.2: Iodide levels in extracts of forages of cattle in μg/kg by natural agro-ecological region in Zimbabwe, 1997.

Natural Region	Sample type	Number of samples	Mean iodide concentration in μg/kg ± standard deviation	Median iodide levels in μg/kg.	Square root transformed geometric mean μg/kg ± standard deviation	Square root transformed iodide geometric median μg/kg
2a	Grass leaves	5 4	20.1 ± 41.47 19.9 ± 34.48	0.69 8.58	2.8 ± 3.91 3.2 ± 3.63	0.82 2.36
2b	Grass Leaves Fruits	16 15 3	5.4 ± 8.47 5.6 ± 6.98 1.1 ± 0.33	2.39 1.44 1.09	1.9 ± 1.35 2.0 ± 1.36 1.1 ± 0.15	1.54 1.19 1.04
3	Grass Leaves	13 1	3.3 ± 4.86 0.5 ± na*	1.31 0.48	1.5 ± 0.97 0.7 ± na*	1.14 0.69
4	Grass Leaves Fruits	10 11 4	69.1 ± 209.95 43.6 ± 87.25 4.0 ± 5.91	1.86 6.05 1.35	3.7 ± 7.83 4.3 ± 5.28 1.6 ± 1.34	1.33 2.45 1.10
5	Grass Leaves Fruits	7 8 2	0.8 ± 0.37 7.1 ± 17.49 42.9 ± 60.11	0.77 0.58 42.90	0.9 ± 0.20 1.7 ± 2.23 4.9 ± 6.08	0.87 0.76 4.93

*na= not applicable

A statistical pairwise comparison of the transformed regional means (Table 4.3) showed that region 4 was not different from region 2a, but had a significantly

(p=0.026) higher mean than regions 2b, 3 and 5. Region 2a however, did not differ significantly with the other three regions. The lowest transformed mean is that for region 3 (1.7 ± 1.03 μg/kg).

Table 4.3 Distribution of iodide levels in forages by natural regions in communal grazing areas in Zimbabwe, 1997.

Natural region	Number of samples tested	Forage iodide means (μg/kg.) ± standard deviation	Square root transformed mean iodide concentration (μg/kg) ± standard deviation	Square root transformed median concentration. (μg/kg.)
2a	9	14.9 ± 28.36	2.6 ± 2.95[1, 2]	1.30
2b	34	4.5 ± 6.83	1.7 ± 1.22[1]	1.38
3	14	3.8 ± 4.83	1.7 ± 1.03[1]	1.76
4	25	40.8 ± 110.39	3.7 ± 5.27[2]	2.18
5	17	8.1 ± 19.82	1.8 ± 2.26[1]	0.66

Natural region geometric means with different superscripts are significantly different ($p<0.05$).

While data normalisation with transformation scaled down the readings, it did not change the relationships among the sample means. The pattern of medians however changed, showing a gradual increase for the higher altitude areas (region 2a) through 2b, 3 to 4 (Table 4.3). The bulk of the iodine was in the grasses (Table 4.2).

The overall means for grasses, fruits and leaves were 18.15, 11.7 and 18.00 respectively, corresponding to transformed means of 2.12, 2.17 and 2.64 respectively

(Table 4.1). There were no significant differences among the different forage types within a region ($p>0.30$). However, when forages were considered together and compared between regions, the levels appeared to increase from the higher lying areas (region 2b) to region 4 which is generally more low lying. This was against high variability with large standard deviations (Table 4.3). Such large variations make in-depth statistical analysis less meaningful. Secondly, region 2a and 3 lacked representation of fruit and forages.

DISCUSSION

McDowell et al., (1986) state that forage mineral analyses are preferable to soil analyses as plants withdraw essential elements from the soil both for themselves and in the process avail elements to meet the needs of foraging animals. This is apart from the difficulty in estimating intake and digestibility in addition to the interaction with other nutrients and the contaminating effect of soils.

In the literature reviewed, the ISE method is preferred for its usefulness for liquid matrices especially milk where direct measurements of free iodide can be estimated (Bruhn and Franke, 1978). Feed materials however require processing involving heating and chemical digestion in which case the result is a measurement of total iodide content including that freed from binding to organic materials. The procedures of sample drying, ashing and dissolution could however result in reduction of iodide levels, especially the initial process of dehydration. Pasteurisation of milk at above 71.5^0C has been noted to increase response readings due to the release of sulfhydryl radical groups in it (La Croix and Wong, 1980). This effect needs evaluation for solid phase samples like feeds.

In this study, blanks included in the ISE procedure alongside the test samples did not provide evidence of iodide volatilisation. This is because no iodide was detected in them. While measurement in milk estimate free iodide ions, pre-treatment of solids renders all iodine in them measurable. Total iodine in pasture materials, rather than free iodide was therefore estimated, but the process of digestion can similarly be expected to free most of the iodide in feeds.

In the sampling, it was not possible to collect the exact same sample in each area due to varying ecological situations. Sample types collected in the field were therefore not homogeneous as they varied with ecosystem, and may not have represented each species adequately.

The sample base being biologically heterogeneous, when disaggregated the sample was also rather too small in some cases, to allow for meaningful factor analysis (Table 4.2), hence the large standard deviations. That readings were obtained however, implied that there is potential in the use of the ISE for solid phase samples. The method requires careful standardization because of the huge variability occurring as indicated by the large standard deviations. This variability may however be indicative of the natural differences in types of plants or the microenvironments in which the forage materials grow. Secondly, the iodide levels did not follow normal distribution patterns and this called for data transformation to permit comparison of means. Normalization of data by transformation makes comparisons possible using parametric statistical procedures and, permits generalisations to be made under that situation. Skewed distributions appear to characterise micro-element levels in biological materials as observed with selenium distribution in pasture materials from comparable areas in a previous study (Ushewokunze-Obatolu, 1988). Transformation might suggest a more logical relationship according to known facts relating to the

effects of altitude (McDowell et al., 1984b). The highest levels still characterised region 4, while region 5 had the lowest (Table 4.3). It will have to be decided if in routine diagnostics, transformation as performed in this study will be of value in assessing individual forage materials.

Using both the raw and transformed means, Region 4 had the highest overall medians. Region 2a also had relatively high means. High pasture iodide content appears "congruent" to higher FT_4 levels in region 2a (Chapter III) in the higher lying areas. In the drier and hotter region 4, pasture iodide was by contrast related to lower FT_4 levels (Chapter III). In theory, a positive relationship in region 4 was expected as the only function of iodine in the mammalian body is to make thyroid hormones (Graham, 1991). This would however depend on availability especially as dietary goitrogens can effectively reduce this. Thus while iodine levels in forages may explain the higher FT_4 levels found in cattle in region 2a (Chapter III), they appear to be a contradiction to the lower FT_4 levels in region 4. It is noted that Matebeleland South province had been singled out as one area with the least incidence of human goiter (Mutamba, 1993). This will however depend on the specific points sampled. In the present study, the two points sampled in Matebeleland South namely Gwanda and Kezi virtually fell in regions 3 and 4. Low FT_4 levels in cattle are most likely to be due to other factors such as goitrogens or interactions with other minerals. This phenomenon could also be due to conversion of FT_4 to FT_3, indicating higher metabolic rate and hence fast weight gains due to more efficient partitioning of nitrogen for muscle building. It should however be recalled that hypo-thyroidism can equally result from too much iodine (Mulei and Daniel,1988; Corah and Ives, 1991). The geometric means of iodide levels given in Table 4.3 ranging between 1.65 and 3.68 μg/kg, could be taken to represent the cold, dry season levels, referring to the

time of sampling. The corresponding untransformed levels at their highest (41 μg/kg or parts per billion (ppb)) in natural region 4 mean that an average animal would need to consume at least 1000 kg of grass in a day in order to meet the daily upper minimum requirement of at least 30mg per animal per day (Olson et al., 1984). This level of feed is unrealistic. Underwood (1971) indicates that hays, straws and common pasture species normally contain levels above 300ppb. At these low levels of total iodine content, forages are approximately 10 to 100 times lower than the optimum concentrations (La Croix and Wong, 1980; NRC, 1984). This is also in the background that roughages may contain more than cereals and oil-seed meals, and that most tropical forages have lower iodine in the dry season (Underwood, 1971). Having been sampled during the dry season, these levels may therefore be the lowest. Existing recommendations to supplement iodine to livestock may therefore be sound and could be important especially for high production systems such as dairying.

It can therefore be said that if the levels recommended in literature are universally correct, the levels of environmental iodine in Zimbabwe are too low and they explain the human endemic goitre problem. Presumably therefore iodine supplementation could result in improved performance in livestock, due to enhanced growth rate and metabolism, provided there are no goitrogens in feeds and the genetic make-up do not predispose animals to goitre.

CONCLUSION

The ISE method detects iodide ions in solid animal feed materials. The large variations recorded in this study point to the need for further standardisation of this

method so as to produce sensitivity values relative to other methods such as the atomic absorptiometry and the gas-liquid chromatography.

Pasture materials in Zimbabwe appear to be grossly deficient of iodine and may cause endemic deficiency which needs correction. This is against a background of variations of serum FT_4 in which the hormone is highest in natural regions where pasture iodine is lowest, with the exception of regions 2a and perhaps region 5. There remains a need to further charasterise the component forages for their contribution to the dietary iodine needs of ruminant livestock using adequately repreentative sample sizes.