CHAPTER II

LITERATURE REVIEW

Physico-chemical properties

Iodine is a black solid with a slight metallic lustre. It is a covalent element belonging to the halogen group (Cotton and Wilkinson, 1976). It sublimes without melting at atmospheric pressure. Iodine readily dissolves in non-polar solvents such as carbon tetrachloride producing a purple vapour. In polar solvents, such as liquid sulphur dioxide, a weak pink complex is formed. Iodine also complexes with starch producing a blue colour. Iodine oxidises easily to form inorganic ions with a wide range of cations (Cotton and Wilkinson, 1976).

Factors affecting iodine availability in feeds

The importance of iodine in nutrition is recognised in many parts of the world due to associated deficiency problems in both humans and animals (McDowell et al., 1984a). In early studies in Great Britain (Orr and Leitch, 1929, as reviewed in Jamieson et al., 1945), it was generally believed that the occurrence of congenital goitre in animals followed closely that in humans. Iodine is known to occur abundantly in the seas, and at lower levels in inland areas. Particularly, seawater weeds and organisms such as seafish and shellfish are major sources of iodine (ARC Report, 1980). The amount of iodine in fresh water reflects its levels in the native rock soils of some areas. Thus, water can be an important source of iodine in some areas (Underwood, 1971). However, Berg et al., (1988), in a study in Illinois USA, reported that the contribution

of iodine in water to levels in pasture was negligible. Highland areas are believed to be relatively deficient due to the effects of leaching and weathering of soils (McDowell et al., 1984b). Studies by Underwood (1971) indicated that while roughages generally contain more iodine than cereals and oil seed meals, tropical forages generally contain less minerals during the dry season. The same author also noted seasonal variations of iodine levels in some species of the iodine-rich seaweeds. Seasonally, lower levels of iodine in milk were reported in summer than in winter (Alderman and Stranks, 1967). These seasonal impacts were attributed to differences in rainfall in various regions of the world (McDowell et al., (1984b). Azoulas and Caple (1984) in Australia for example, found that levels of milk iodine were highest at the end of summer and lowest at the end of spring.

In Zimbabwe, studies showed that hypothyroidism occurred in sheep that grazed on star grass (*Cynodon aethiopicus*) pastures that were heavily fertilised with nitrogen and phosphates (Rodel, 1971; Rudert and O'Donovan, 1974; Rudert and Oliver, 1978). In communal areas, grazing areas are separate from cropping areas. Grazing areas in this sector are generally never fertilised. Crops are harvested between May and July, following which livestock may be allowed into these agricultural lands to forage on residues. The effects of this communal management practice on iodine has not been studied before.

Hypothyroidism can also result from goitrins and alyphatic disulphides found in garlic and onions which interfere with the metabolic pathway by inhibiting the function of thyroperoxidase enzyme in the formation of thyroid hormone (Underwood, 1971). Certain feeds such as maize silage and soya meal fed to in-calf cows have been shown to lead to goitrous calves (Hemken, 1970). Use of iodine containing disinfectants as

teat dips, for wounds and uterine lavages may raise the blood levels of iodine (Sertl and Malone, 1993).

Factors affecting iodine requirements in animals

Bovine foetuses may have over 5 times the plasma concentration of iodine compared to the dams, due to inability of the foetus to excrete iodine in urine (Miller et al., 1967). This situation may however, be crucial for neonatal survival. Iodine levels are affected by age where young animals benefit from higher concentrations of iodine in milk (Miller et al., 1975). Lactating and suckling dams therefore lose larger amounts of iodine than their dry counterparts. Human females including those at pre-pubertal phases are more likely to have lower iodine levels compared to their male counterparts (Dent et al., 1968), although with advancing age, both sexes are affected (Ridgway, 1996). By extrapolation, sex differences could imply that because of increased losses due to lactation (Franke et al., 1983; Berg et al., 1988), females of animal species might be at constant risk of hypothyroidism. Sex and stage of lactation therefore influence levels of iodine (Franke et al.,1983). In the latter factor, cows in late lactational period usually have higher milk iodine.

Seasonal effects with high winter levels, some due to concentrate feeding have been noted, where cow milk iodine levels in winter ranged between 50 and 150μg/litre due to intake of 20 - 50mg/cow/day as opposed to <5mg/cow/day in summer (Alderman and Stranks, 1967) Further, low levels of iodine have been reported during high environmental temperatures (Andrewartha et al., 1980).

Thus, Iodine requirements fall with rise in ambient temperature due to reduced need to generate body heat through metabolism (Yousef and Johnson, 1966; Sutherland and Irvine, 1974).

Breed affects iodine levels as evidenced by the hereditary goitre which occurs in less than 1% of Afrikander cattle (Ricketts and Van den plas, 1990). In these cows, this hereditary goitre has been found to be due to a point mutation in the thyroglobulin gene (Ricketts et al., 1987).

Health problems associated with iodine levels

Endemic iodine deficiency is one of the most prevalent and serious deficiency conditions of man and livestock world wide (McDowell and Conrad, 1989). Iodine deficiency can co-exist with that of other minerals such as calcium, magnesium, zinc, selenium, among others, thus increasing the negative effects on growth (Jooste et al., 1994; Arthur et al., 1999; Underwood and Suttle, 2001). These effects have been recognised as major problems in human populations in Zimbabwe (Dent et al., 1968; Whittle and Walker, 1968) and Zambia (Onushko, 1975; Wenlock, 1975).

The singular function of iodine in the mammalian body is in the biosynthesis of thyroid hormones (Graham, 1991). Clinically, iodine deficiency is assumed when the thyroid gland becomes enlarged resulting in condition known as goitre. Nevertheless, goitre may not be the most sensitive indicator of iodine deficiency (Andrewartha et al., 1980), but it is a final common expression of separate disease processes, which according to Underwood (1971), are: 1) a primary dietary deficiency of iodine which is associated with its low levels in feed, water and soil; 2) deficiency of iodine caused

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by goitrogens of various types leading to defective hormonogenesis; and 3) metabolic disability including genetic effects leading to abnormal hormone formation (Pammenter et al., 1978; De Vijdler et al., 1978; Schulz and Groenewald, 1983; Ricketts et al., 1985; Arthur et al., 1988; Welchman et al., 1994). Endemic goitre can also result from prolonged consumption of high levels of iodine, which leads to a severe reduction of iodine uptake by the thyroid (Fish and Swanson, 1983).

Iodine deficiency and goitrogens lead to reduced basal metabolic rate and thermo-regulation, muscle weakness, reduced growth, decreased cardiac output, reduced myocardial contractility, alterations in skin and hair formation and reduced apocrine secretions (Underwood, 1971). Effects of iodine on reproductive function include impairment of embryogenesis, bone development and infertility (Hemken, 1970). High incidences of abortions, stillborn and weak calves have been noted in iodine deficiency herds (Allcroft et al., 1954). A resultant thyroxine deficiency in lambs leads to poor cold resistance and death in adverse weather conditions and twin lambs are at greater risk than singles (Alexander et al., 1990). Hypothyroidism, including that caused by thyrotoxicosis (Olson et al., 1984), can also lead to depressed adrenocortical functions (Robertson et al., 1957) resulting in ketosis with significant effects in milking cattle (Hillman and Curtis, 1979). Therefore, iodine deficiency in animals causes reduction in levels of production and productivity. Economically, these effects could be highly significant in iodine deficiency endemic situations thus, justifying diagnostic investigation for differentiation purposes, for the application of corrective interventions.

Thyroxin deficiency caused by metabolic goitrins cannot be reversed by dietary iodine supplementation but by alteration of the feed base to remove the goitrogenous

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materials (ARC Report, 1980). Similarly, impairment of conversion of T_4 to T_3 caused by selenium deficiency in rats (Beckett et al., 1987) and cattle (Arthur et al., 1988), cannot simply be corrected by supplementing iodine. Simple, thiocyanate-induced hypothyroidism can be on the other hand, be reversed by dietary supplementation with iodine (Hemken et al., 1971).

Excess iodine, oestrogens and lithium, as well as hypomagnesemia, can impair thyroid hormone synthesis, release and peripheral metabolism (Mulei and Daniel, 1988; Gaitan and Cooksey, 1989 quoted by Graham, 1991). Iodine toxicity, which can lead to symptoms of deficiency occurs in cattle receiving more than 50mg/day (Hillman and Curtis, 1980; Olson et al., 1984; Corah and Ives, 1991), which respresents 2 to 20 times the recommended daily intake per animal. Threshold toxicity levels may be elevated by the level of exposure to goitrogens, or reduced by use of iodine-based disinfectants (Graham, 1991). Chronic excessive iodine intake blocks thyroid hormone synthesis by inhibiting the enzymes involved in hormone biosynthesis, resulting in reduced thyroxine secretion, and hypothyroidism (Roti and Vagenakis, 1991).

Metabolism and function of Iodine

Iodine affects metabolism, growth and differentiation in all mammals and birds (Underwood, 1971). It also affects practically all organs at some stage of growth and maturation (Graham, 1991). The expressed effects are organ and tissue-specific and include neuromuscular function, reproduction, glandular and gonadal interactions, epithelial maturation, bone development, oxygen consumption and cardiac output and function (Underwood, 1971; Graham, 1991).

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According to Hemken (1970), Bustad and Fuller (1970), and review by Miller et al.,
(1975), 70-90% iodine is absorbed as an iodide in the rumen, reticulum and omasum
while, 10% is absorbed in the abomasum (Barua et al., 1964). At the same time, the
abomasum is able to concentrate iodine and secrete it at 18 times the absorption rate,
to be reabsorbed in the small intestine and the rest of the gastrointestinal tract (Miller
et al., 1975). Absorbed iodine exists in circulation as protein bound iodine (PBI) or as
free inorganic iodide, and is concentrated primarily in the thyroid gland where it is
converted into the thyroid hormones, 3,5,3'5'-tetraiodothyronine (thyroxine or T_4) and
3,5,3'- triiodothyronine   (T_3) (ARC Report, 1980; Graham, 1991).   The thyroid
normally contains iodine content equal to the daily dietary intake (Miller et al., 1975).

Fig 2.1: Biochemical structure of T_3 and T_4. Source: Waters, The Liquid Chromatography People, Milford, Massachusettes, 01757, USA.

Thyroid Hormones

Triiodothyronine (T_3)

Tetraiodothyronine T_4)
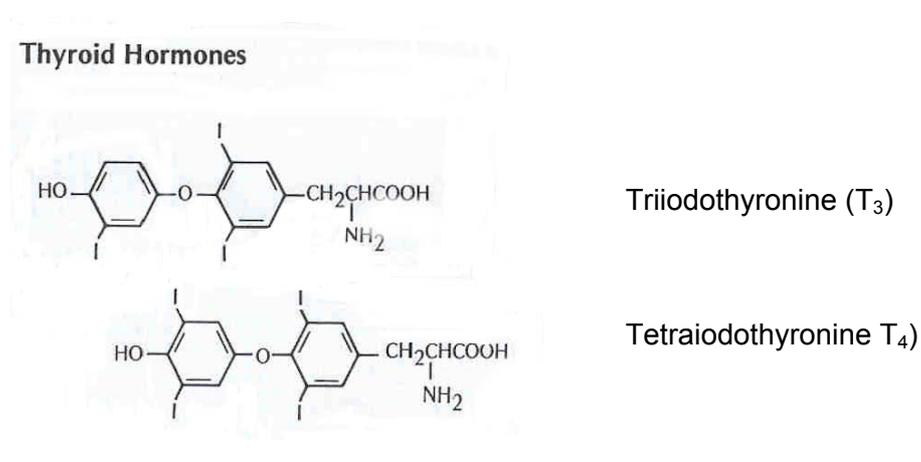
T_3 and T_4 are lipophilic and they circulate in blood mainly bound to thyroxine binding protein (TBG, 70%), thyroxin binding prealbumin (5-20%), albumin (10-25%) and other proteins (Irvine, 1969; Refetoff and Larsen, 1989 cited by Graham, 1991). Unlike T_4, considerably less T_3 binds to T_4-binding pre-albumin (Klee, 1996). A feedback mechanism between the hypothalamus and thyroid function develops early in foetal life (Wolff et al., 1949) and utilises iodide that is readily transferred transplacentally (Miller et al., 1967). This is developmentally advantageous to foetal health. About 85% of T_3 which is the utilisable form in circulation, is generated in the liver through deiodination of T_4 (Kahl et al., 1984; Graham, 1991; Ferguson, 1994). The rest is produced peripherally in tissues like the brain and brown adipose tissue, to act locally (Fisher, 1996). The pituitary controls the concentrations of T_3 and T_4, affecting modification, synthesis and release through thyrotropin (thyroid stimulating hormone or TSH) (Arthur et al., 1990). TSH release from the pituitary is regulated in turn by the thyroid releasing hormone (TRH) from the hypophysis and somatostatin levels, as well as levels of T_4 and T_3 (Graham, 1991; Kohrle, 1989). However, it is worth noting that the rate of thyroid hormone secretion in cattle is inversely related to ambient temperature (Yousef and Johnson, 1966 and 1968; ARC Report, 1980) which is probably responsible for the low summer levels of this hormone. While in most cases of hyperthyroidism, T_3 correlates with T_4 concentrations, occasionally, T_3 is the only hormone increased, leading to T_3 thyrotoxicosis (Klee, 1996).

Forty percent of iodine is excreted through urine, while 30% is lost through faeces (Graham, 1991). Excretion through milk is related to dietary intake in cattle

(Alderman and Stranks, 1967; Hemken, 1970; Miller et al., 1975; Franke et al., 1983).

Milk iodine in herd bulk milk is directly and highly (92%) correlated to dietary intake (Miller et al., 1975), although significant individual variations may exist (Berg et al., 1988). In lactating cows, 8% of iodine is excreted through the milk, compared with 40% in ewes (Miller et al., 1975), and losses through milk are directly related to milk yields. Therefore, lactation can lead to heavy losses of iodine in high producing lactating animals (Miller et al., 1975). Levels of dietary iodine and stage of lactation therefore affect iodine levels in milk (Swanson, 1972), a major source of iodine to the young. Milk supplies about 42% of dietary iodine for humans, when iodised salt is excluded (Kidd et al., 1974). The iodine levels of milk in goitrous areas are less than 80µg/litre for sheep and 20µg/litre for cattle (Alderman and Stranks, 1967; Mason, 1976). For cattle, milk levels lower than 10µg/litre are generally considered deficient, while those above 25µg/litre are regarded as sufficient (Binnerts, 1954).

Thiocyanates found in cassava and brassicae (Barry et al., 1983; Reid et al., 1994), act by altering trans-membrane transfer of iodide, inhibiting iodide retention in the thyroid and its incorporation into thyronine. Metabolic goitrogens on the other hand, act by inhibiting thyroperoxidase, preventing the formation of mono- and di-iodotyrosine, the thyroid hormone precursors (Underwood, 1971). According to Ermans and Bourdoux 1989, quoted by Graham, 1991, metabolic goitrins also inhibit the conversion of T_4 to T_3. Both these effects lead to goitre. Increase in T_4 may result in increased growth rate and energy deposition (Rumsey et al., 1990).

A point mutation on the thyroid hormone gene leads to a heritable trait which causes excessive production of diiodotyrosine (DIT), a precursor of thyroid hormones (Van Zyl

et al., 1965), at the expense of the functional thyroid hormone. The mutation causes the restriction enzyme Taq-I to lose its active site by replacing cytosine with thymine, resulting in the generation of a 1900-base pair (bp) instead of a 1500-bp fragment (Ricketts et al., 1985; 1987). This creation results in a partial thyroglobulin molecule that is not effective metabolically. Asymptomatic heterozygous carriers can exist in which both fragment types are detected following enzyme action (Ricketts and Van den plas, 1990). This is seen as an inefficient and defective pathway for thyroid hormone sysnthesis (Van Jaarsveld et al., 1972). A high proportion of serum iodine in the marginally euthyroid, but goitrous animals, is due to iodoalbumin with free T_3, T_4 and DIT contributing only partially to the levels of protein bound iodine (Ricketts et al., 1985).

Deficiency of selenium has also been shown to be associated with an increase in creatinine and plasma urea and a decrease in alkaline phosphatase, all of which occur in hypothyroidism (Arthur et al., 1999). This indicates that some effects of selenium deficiency, including the effect of selenium on growth, are mediated through a resultant alteration in thyroid hormone metabolism (Arthur et al., 1988). On the other hand, Growth Hormone potentiates T_4 deiodination into T_3 by increasing extrathyroidal tissue monodeiodinase (Rumsey et al., 1990).

Methods of supplementation

Iodine is highly absorbable as an element (Graham,1991). Topical applications on the skin (as in udder washes), of iodine-containing soaps and disinfectants result in the absorption of this element (Hemken, 1980). Practising veterinarians often

recommend this method of supplementation especially in pigs as a treatment for goitre. A wide range of inorganic iodide compounds, such as sodium or potassium iodide and calcium iodate are recommended for use as salt licks (Rudert and O'Donovan, 1974). However, unstabilised potassium iodide is highly volatile or easily leached and may therefore be less effective under wet tropical conditions (ARC Report, 1980). Iodates and more complex organic forms such as penta-calcium orthoperiodate and ethylene diamine-dihydroiodide (EDDI) are strongly recommended as sources of iodine supplements for cattle since they are more stable and are less vulnerable to leaching and volatilisation (McDowell et al., 1984a). Addition of as little as 4ppm of EDDI in dairy concentrate has been shown to lead to high increases in milk iodine especially in late lactation (Franke et al., 1983).

Supplementation can be direct through water, mineral licks, mixtures, drenches, rumenal pellets or bullets and injectables (Ammerman and Miller, 1972; Dunn and Haar, 1990). Thus supplementation can be given by dosage or in dietary formulations *ad-libitum*. Supplementation can also be indirect through the use of mineral-containing pasture fertilisers. It can also be achieved by alteration of soil pH in pastures or growing specific pasture species which concentrate iodine (McDowell et al., 1984a; McDowell and Conrad, 1990).

Methods for the determination of iodine status in animals

Assessment of iodine status is a requirement for the confirmation of associated health problems (Azoulas and Caple 1984; Kaufmann et al., 1998). Confirmation based on quantitative studies is necessary in assisting producers in making decisions about iodine in nutritional management. It also helps in the monitoring of herd health and

productivity of animal enterprises, and as a tool in preventive medicine and public health management (Dent et al., 1968; Berg et al., 1988). Some conditions in which iodine or thyroid hormone status is required include thyrotoxicosis, hypothyroidism, assessment of supplementation programmes, assessment of hereditary and environmental effects as well as in the differential diagnosis of a wide variety of sub-clinical conditions. This is in light of the importance of iodine nutrition for animal productivity, and animals being a source of iodine in human nutrition, especially through milk (Phillips, 1997).

As iodine is important in metabolism through thyroid hormones, it has influences on the quality of meat (Borger and Davis, 1974), an essential requirement in domestic and international trade. The tendency by commercial livestock producers is to use thyroid suppressing drugs such as thiouracil and its derivatives which may affect meat through slowing down of metabolism by reducing calorigenesis (Stasilli et al., 1960; Rumsey et al., 1985a; 1985b; 1988), possibly enhancing weight gain and causing lacing of muscle with adipose tissue. In the small-holder sector, hypothyroidism can only occur naturally due to exposure to either environmental goitrogens, toxic or very low levels of iodine in natural dietary sources.

Thus, confirmation of iodine status should be a desired necessity by both small- and large-scale farmers as well as animal and human health practitioners.

Preferred routine test procedures must be those that enable diagnosis both at individual and herd levels. They must therefore meet the conditions of low cost, ease, efficiency, reproducibility, accuracy, suitability for herd measurements, robustness and must give quantitative results to enable decisions on interventions to be accurately made. Another determinant of a method of choice is the type of sample on which the test is applied.

Indirect approaches to estimate iodine levels in animals

Although the best way to confirm mineral imbalances or deficiencies is through experimental supplementation and dose-response assessment (McDowell et al., 1986; McDowell and Conrad, 1989; McDowell, et al., 1993), costs in time, and borderline deficiencies which do not cause overt deficiency symptoms make this method unreliable. Alternative methods of assaying iodine levels in feeds, water, soil and a variety of animal samples can be applied. However, results from such analyses may be difficult to interpret. For example, the uptake of a mineral by plants may not be directly related to either its levels in soil or in animal body samples. This could be due to its relative availability or to plant factors such as maturity, ecotype and species. Analysis of soluble soil extracts can be used to assess available forms although they too can be unreliable as soils are normally consumed inadvertently.

Forages, while being better for analysis than soils are prone to soil contamination and may not represent what the animals eat as well as presenting a difficulty in estimating intake and high variability in forage element content (McDowell et al., 1986). The soil levels of iodine are usually higher than in forage such that effects of rain splashes may result in over-estimation (Healy, 1974; McDowell et al., 1986). Another problem in forage sampling is that of assuring representativeness, where only a very small proportion, including leaves and bark, may form the diet (Leigh and Mulham, 1966). In addition, forage intake and digestibility in animals may be difficult to estimate (McDowell, et al., 1983; McDowell et al., 1986).

Reconnaissance mapping of large areas has been postulated to be immensely useful for planning nutritional management of animals (McDowell and Conrad, 1989). Some workers have attempted regional geochemical surveys, the results of which remain equivocal (Mills, 1996; Plant et al., 1996; Meyer et al., 2000). However, these studies conducted in Zimbabwe using stream sediments and forages did not include iodine (Fordyce et al., 1996).

Analyses of animal tissues and fluids attempt to assess the contribution of the total environment in meeting the animal needs, and as such are important in confirming influences of environmental factors in determining dietary levels of a particular mineral (McDowell et al., 1986).

Direct methods

Gravimetric method

Due to the difficulty of applying visual assessment of goitre in animals as used in human clinical diagnosis of iodine deficiency, the gravimetric method is applied at necropsy (ARC Report, 1980). This is done by weighing the thyroid gland and comparing this weight with the crown to rump lengths of the foetus or newborn to assess congenital goitre (Wolff et al., 1949). A linear logarithmic relationship between these measures has been reported (Wolff et al., 1949). Assessment can also be made by comparing thyroid glands of normal and suspect animals. It has been reported that a 2-fold increase in gland weight suggests iodine deficiency (Allcroft et al., 1954). Generally, normal weights of foetal thyroid glands at term range between 6 and 6.6g (Jamieson et al., 1945; Nichols et al., 1949). There is a linear relationship between thyroid weights and T_4 concentrations in lambs (Alexander et al., 1990). In addition,

confirmation of hypothyroidism can be done by observing reduced colloid and hyperplasia of the thyroid histopathologically (Herenda and Dukes, 1989).

Physical measurements (palpation and weighing) of the thyroid gland are practical and useful for rapid diagnosis including assessments of borderline deficiency cases (Dent et al., 1968). Weight measurements may be unreliable as thyroid weight may also increase as a result of high iodine levels in feeds (Corah and Ives, 1991) as reported in horses (Baker and Lindsey, 1968) and humans (Wolff, 1969). Further, weight measurements are likely to be affected by other conditions such as oedema, congestion and fat that can cause large variations in diagnostic outcomes and decisions. In addition, such a method would require the establishment of a standard curve thereby calling for large sample sizes for given circumstances to use in assessing cases. Specific studies are required, based on sufficient numbers of dead animals under various circumstances which would be used to make a firm diagnosis. Clearly, standardisation would be a problem.

Iodine estimation

The amount of thyroid hormone produced depends on the iodine status of the animal (Berg et al., 1988). In the body, the highest concentration of iodine is in the thyroid gland where thyrohormone synthesis takes place (ARC Report, 1980). Iodine can be assayed in milk and urine, where it occurs as iodide and in serum where it occurs conjugated mainly to globulins, albumin, pre-albumins and post-albumin (Oppenheimer et al., 1963; Osorio, 1967; Grace, 1995). Assays measure the levels of protein bound or free iodine. Iodine can occur in both these forms either as hormonal iodine or as iodide ions (Refetoff et al., 1970). In serum analysis, the first step is therefore to effect separation of iodides and iodine by precipitation or elution (McDowell et al., 1984b).

Solid matrix samples presumed to contain iodide can be dry or wet-ashed in alkali to remove the bulk of organic material before assaying. Ashing is achieved at not more than 450^0C to limit losses due to volatilisation (Binnerts and Das, 1974). Subsequent to pre-treatment, biochemical determination of physiological iodine levels is achieved by measurement of its catalytic action as iodide. Iodide catalyses the oxidation reaction of arsenious oxide by ceric sulphate (Ce-As-I), in an atmosphere free of trace contamination by iodine and mercury (Osorio, 1967). The process begins with conversion of iodide by oxidation to iodate, followed by reduction to iodine. The amount of iodine present is estimated colorimetrically by photometry or titration (Binnerts and Das, 1974; Mason, 1976). These techniques are now available in automated form (Leonards and Davoren, undated laboratory manual; Dunn and Haar, 1990).

Low precision, poor reproducibility and lengthiness have been reported to affect the Ce-As-I catalytic as well as the neutron activation methods for iodide ions (Fischer et al., 1986). This is because of the many stages involved, which make the methods tedious, expensive and, reproducibility may be limited, and recovery low (Fischer et al., 1986). In addition, the atmosphere must be free from iodine and mercury which interfere with analysis, the latter resulting in low readings (Osorio, 1967). However, where automation is practical, these problems can be minimised.

Iodine assays may also include iodide of no biological value such as that bound to drugs (Binnerts and Das, 1974). Species differences, however do occur, and these need to be taken into account since protein binding appears to be species-linked (Farer et al.,1961) and physiologically dependant (Irvine, 1967a; Refetoff et al., 1970). Differences in

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protein binding have been found between humans and rhesus monkeys on the one hand and artiodactyla (cloven hoofed animals) on the other (Refetoff et al., 1970).

A radioactivation analysis described by Ohno (1980) is highly suitable to liquid samples of large volumes such as milk. In this highly sensitive technique, the iodine in the sample is activated by either thermal neutron reaction or a resonance neutron reaction to form radio-nuclides, mainly I^{127} (Binnerts and Das, 1974). The activated samples are then extracted by an anion exchange procedure and through a system of solvents to separate the radioactive iodine, which is then assayed by gamma ray spectrometry or beta counting in a Geiger-Muller counter (Binnerts and Das, 1974).

Apart from the need to move away from radioisotope use on grounds of safety, the main disadvantages of the radioactivation method are their high cost and the need for a nuclear reactor (Heckman, 1979). Furthermore, the presence of chlorine and bromine can introduce false readings in radio-assays (Rook, 1977), and this calls for additional steps in separation (Binnerts and Das, 1974).

Radiollabelled iodine can also be fed to experimental animals to estimate thyroid uptake and urinary excretion which give estimates of iodine requirements. In hypothyroidic individuals, the uptake rate is higher while the urinary excretion rate is lower, whereas the opposite is expected in euthyroid or hyperthyroid individuals (Buzina et al., 1959).

Gas chromatography (GC) is another method for estimating iodine which is described by Naumann, (1967). Gas chromatographic methods using ^{63}Ni electron capture to estimate thyrohormone in serum described by Naumman et al., (1967) and Sterling et al., (1969) are based on iodine estimation.

Improvements have been made by others, resulting in a liquid chromatography (LC) method as described by Sertl and Malone (1993), in which a liquid sample such as milk is first filtered using a membrane to remove all solids. The resulting clear fluid is then injected into the LC column for separation of the iodide using reversed phase ion-pair chromatography with a dc amperometry detector (Murphy and Jachan, 1965). Gas chromatographic techniques using [63] Ni electron capture are likely to involve tedious extraction procedures on each sample and replicate, as the final stages are preceded by dialysis and column chromatography (Sterling et al., 1969; Naumann et al., 1967). The present author's experience is that standardisation in Gas liquid Chromatography can be difficult and detector systems can be affected by ambient conditions. While liquid chromatography has been found to be a reliable and sensitive technique for quantifying iodine in milk, it may not be specific enough as it picks up other halogens in volatile compounds (Rook, 1977).

An ion selective electrode (ISE) method which can specifically detect the iodide ions by measuring the total electric potential on a pH meter with an expanded scale has also been found to be of practical value for liquids, particularly milk (Bruhn and Franke, 1978). Miles (1978) found this method to be the simplest and fastest for milk iodide. Berg et al., (1988) and Maas et al., (1989) found an 81% correlation between dietary and serum iodine using the ISE. The ISE method could however be overly sensitive for lower iodide concentrations while, being poorly sensitive for higher concentrations (Bruhn and Franke, 1978). While the ISE method picks only the iodide ions in milk, the chemical method because of the inherent conversion of iodine to iodide, detects total iodine levels (Bruhn an Franke,1978). According to Bruhn and Franke (1978) false positive readings occur with the ISE method because it picks

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sulfhydryl groups from sulphur-containing amino acids as well, particularly when iodide concentrations are below 300μg /kg milk. The test tends to perform optimally between 300 and 700μg/kg milk iodine. Further, these authors report that at concentrations higher than 700μg /kg, most iodine is in the organic form, and the electrodes give lower readings and that milk fat deposits also reduce electrode sensitivity. Heating of milk leads to an apparent increase in iodine concentration although conventional pasteurisation accepted in terms of time-temperature conditions required for public health, does not have this effect (LaCroix and Wong, 1980). Other halogens interfere, depending on the solubility of their silver compound deposits (Rook, 1977). The ISE method therefore has limited application for accurate assays. However, by comparison with the chemical method, Bruhn and Franke (1978) showed that the ISE method was an acceptably rapid, relatively precise and accurate method for measuring milk iodine. In addition, it is easier, less expensive than the chemical and radioactive techniques, and when used without sample pre-treatment, is free from losses due to alkali sample digestion (LaCroix and Wong, 1980). In their study, Bruhn and Franke (1978) concluded that the ISE method was of value in determining husbandry management practices that influence raw milk iodine concentrations.

Thyroxine measurement

As a significant proportion of thyroid hormones binds to serum proteins, total plasma or total serum thyroxine can be estimated by measuring the iodine in precipitated serum proteins, for example PBI, using the methods outlined for iodine in the preceding paragraphs. In thyroxine measurement, iodine precipitating with protein may not always be only due to hormone protein binding but, may also be due to either iodinated protein

for example, thyroglobulin, abnormal iodinated thyroid protein, iodinated albumin or inorganic iodine. Because there is low correlation between PBI and thyroid hormone flux, determination of the functional status of thyroid by estimation of PBI may not be very accurate (Mixner et al., 1962). Acid butanol selectively extracts thyroid hormones from other iodinated proteins (Osorio, 1967). The iodine thus extracted can then be measured by the chemical, ceric oxidation method (Fisher et al., 1964). According to Osorio (1967) when serum butanol extractable iodine (BEI) is significantly lower than PBI, circulating thyroglobulin should be suspected. If on the other hand, total serum iodide exceeds PBI by more than one microgram per 100ml, contamination by iodine containing drugs or iodine-containing contrast media should be suspected.

Sutherland and Irvine (1973) described a technique for assaying total iodothyronines after separation from other plasma components on strong anion-exchange resin columns, followed by chemical determination of iodine through Ce-As catalysis.

Murphy and Jachan (1965) described the determination of total thyroxine in serum, in a procedure procedure that begins with a single ethanol extraction of thyroxin from plasma in the unknown sample. This is followed by competitive protein binding analysis of the T_4, in competition with a fixed amount of radio-labelled thyroxine for binding sites on a fixed amount of thyroxine binding globulin. Separation of bound labelled hormone from the unbound following the competition reaction is achieved by resin precipitation, which these workers also found to be better and less demanding of equipment than column separation. With the competitive binding thyroxin method, (Murphy and Jachan, 1965), large numbers of samples can be processed, and apart from counting for radioactivity, no specialised equipment is required. Unlike PBI determination, this method is not affected by iodine or mercury (Murphy and Jachan, 1965).

The procedure by Murphy and Jachan (1965) appears to be similar to measurement of total serum T_3 and T_4 by saturation analysis using standard serum with thyroxine binding globulin (TBG) referred to by Meinhold and Wenzel (1974), quoting Ekins (1960). In this method, increasing the amount of serum T_3 and T_4, progressively reduces the proportion of hormone bound to TBG and hence the amount of radiolabelled thyrohormone that can be bound. This can be plotted on a standard curve which can then be a basis for reading the estimates (Sterling et al., 1969). Osorio (1967), assesses this procedure as being specific for T_4, devoid of interference by iodide or mercury and fairly easy to carry out.

Thyroid function is more accurately reflected by levels of T_3 and T_4, as these are its main products. The free T_3 (FT$_3$) and free T_4 (FT$_4$) are the biologically active fractions that are responsible for regulating the secretion of TSH by negative feedback, hence they are the most important to separate and measure (Ekins, 1992). Measuring the free hormones is superior to measuring total hormones especially in cases where TBG concentrations are altered, cases where albumin is low and cases with abnormal thyroid hormone binding protein (Klee, 1996). FT$_3$ and FT$_4$ are separated from the bound thyroxine by paper electrophoresis, ion exchange chromatography, gel filtration or erythrocyte uptake (Osorio, 1967). The free hormone fractions can also be estimated by measuring the fractional dialysis rate of radio-labelled hormonal iodine across a semi-permeable membrane (Christensen, 1959, 1960). Other suggested approaches are the use of equilibrium dialysis of diluted serum in an aqueous buffer at pH 7.4, to measure the relative concentration of radio-labelled iodine of free thyroxine, and filter paper electrophoresis of whole serum at pH 8.6 in glycine acetate buffer (Oppenheimmer et al., 1963). Simultaneous uptake of radio-labelled hormonal iodine by erythrocytes and PBI

have also been described to estimate FT_3 and FT_4 (Osorio, 1967). Separation of free and bound hormones can also be achieved by the double antibody technique using rabbit anti-thyrohormone antibodies produced by injecting bovine or human hormone conjugates (Meinhold and Wenzel, 1974).

Eastman et al., (1975) and Ekins, (1982, 1992), gave a full description of the RIA technique for free thyrohormone fractions. Brown et al., (1970) reported the production of T_3 specific antibodies by injecting animals with a condensed product of T_3 and succinyl poly-L-lysine; and for T_4 by using conjugated hapten-protein antigens. A double antibody assay technique has been described by Kahl and Bitman (1983) for estimation of T_3 and T_4. In RIA, it is necessary to employ compounds that limit the binding of T_3 to TBG and hence render T_3 available for reaction with antibody (Eastman et al., 1975). Some such compounds that have been used are thyroxine, Tetrachlor thyronine, salicylate, sulphonic acid and diazepam. The T_4 which can also be used as a competitor in limiting TBG binding of T_3, is likely to be contaminated with T_3, and can undergo spontaneous deiodination to T_3 during incubation and this could distort the readings (Eastmann et al., 1975). Meinhold and Wenzel (1974) found 8-anilinonaphthalene-sulfonic acid to be better in limiting binding to serum proteins, than merthiolate and sodium salicylate.

Other techniques to separate the free hormone from the bound one, such as the use of dextran coated charcoal and ion-exchange resin were found to result in reduced antibody binding in the RIA. (Meinhold and Wenzel, 1974). Such immuno-assays offer measurement options through counting of radioactivity or measuring chemiluminescence in the reacted fractions (Woodhead et al., 1974).

Immunoassays have an advantage in the analysis of free T_4 due to the fact that at physiological pH, the T_4 binds more intensely to serum proteins than T_3 (Osorio,1967). The FT_4 that reflects the thyroidal status is such a very small fraction that cannot practically be measured by chemical techniques (Oppenheimer et al., 1963; Osorio, 1967). FT_4 however, exists in equilibrium with the total T_4 in circulation and hence correlates closely with clinical thyroid status inspite of changes that may affect serum protein concentrations and its nature (Refetoff et al., 1970). These authors also found that in the face of wider interspecies variability in total serum thyroxine, FT_4 was a more accurate indicator of thyroid status. Possible interference from diiodotyrosine (DIT) can be minimised by immuno-precipitation with anti DIT serum (Meinhold et al., 1981).

A variety of RIA test formats for both T_3 and T_4 are now available in kit forms. Their use in studies using human samples showed cross-reactions with DIT of less than 2.5% (Meinhold et al., 1981). In addition, over-estimation of T_4 may occur if the serum has T_4 antibodies, or in rare events of dysalbuminemic hyperthyroxinemia where the albumin's affinity for T_4 is increased (Meinhold et al., 1981). In such situations, T_3 and TSH should also be measured (Bio-Merieux, undated). Within the reference range, a Coat-A-Count free T_4 RIA test maintains high precision levels with an intra-assay coefficient of variation (CV) of about 5% and an inter-assay CV of between 7% and 9% (DPC, 1997).

Underwood (1971) stated that more accurate estimates of iodine status are based on thyroid hormone secretion rates (TSR) using radioactive iodine. This method works on the principle that rates are higher and the half-life shorter in deficiency states as well as in work animals (Irvine, 1967 b). Judging from the nature of the method, it is however valuable only in single-case investigations rather than herd epidemiologic evaluations.

TSH estimations

As circulating thyrotropin (thyroid stimulating hormone or TSH) controls thyroid gland activity, it is also a reliable indicator of iodine status. In humans, thyroid stimulating hormone ELISAs are used to determine thyroxine status where TSH elevation denotes deficiency, and low TSH denotes sufficiency or hyperthyroidism (Nelson and Wilcox, 1996). This ELISA principle is used in a solid phase, two-site fluoro-immunometric assay using two mouse-derived monoclonal antibodies (Spectrascreen, 1992; Kodak, 1992), and is read through chemiluminescence by spectrophotometry. Local attempts to use such a kit test in animals have not yielded reliable results, pointing to the possible need for species specific reagents.

Attempts to utilise RIA tests for measuring TSH levels in animals indicated that the test suffered from low specificity due to cross-reactions of the rabbit produced polyclonal antiserum with luteinising hormone (LH) and follicle stimulating hormone (FSH) (Parlow, 1999). This is a result of similarities in the alpha and ß subunits of TSH with those of the other hormones. These cross reactions could be minimised by Sepharose® immunoadsorption of antisera, preadsorption of antisera with LH and FSH, as well as radioiodination of the ß-subunit of bovine TSH (Borger and Davis, 1974).

For individual evaluation of hypothyroidism, the TSH stimulation test can also be used to test response of serum by T_3 and T_4 levels (Ferguson, 1994) 6 hours after TSH administration. In nature, TSH levels respond only when serum T_4 levels fall to 27% of euthyroid levels (Leung et al., 1976). In hypothyroidism, the response is exaggerated

and prolonged, while it is minimal or negligible in the normal or hyperthyroid state (Ormston, 1972; Seth et al., 1984). Diagnosis of hypothyroidism using T_4 levels that may be difficult in borderline cases, are easier to make using the TSH levels in addition (Ridgway, 1996), where raised levels usually confirm mild conditions, rendering TSH assays more precise and sensitive. TSH levels are known to respond logarithmically to significant arithmetic deviations of FT_4 (Keffer, 1996).   Further, the pituitary reserve of TSH may also be monitored by measuring its levels in response to administered thyrotropin releasing hormone (Ferguson, 1994).   The TSH response is increased in hypothyroidism, while it is decreased in hyperthyroidism (Ferguson, 1994).

Diagnosis of genetic determinants

Southern blots of the thyroglobulin gene can be applied to the isolated DNA, to screen for the mutation (Ricketts et al., 1985). Thyroglobulin gene fragments can be visualised by hybridisation with a ^{32}P- labelled recombinant bovine thyroglobulin cDNA probe. Blood leucocyte DNA is effective for this method (Ricketts and Van den plas, 1990). Others are thyroid or liver tissue (Ricketts et al., 1985). Immunoprecipitation of thyroglobulin with analysis of precipitate by gel electrophoresis in SDS has been described (Pammenter et al., 1978).

At cellular level, electron microscopy of the thyroid cells shows poorly developed endoplasmic reticulum and the presence of more lysosomes of bigger sizes (Pammenter et al., 1978).   In addition, there are increased levels of iodo-albumin in the serum (Robbins et al., 1966).

DISCUSSION

Of the four methods which can be applied in assaying iodine in animals, the most convenient appear to be the ISE and the LC methods. Their main advantage over the catalytic/colorimetric and the radioactivation methods are in their higher potential for simplicity and reproducibility. They both can be used for determining the iodine concentrations in milk and urine samples. The observed levels would indicate the dietary iodine status of herds (Swanson, 1972; Berg et al., 1988; Trávnícek and Kursa, 2001). Used on feeds however, the advantages of the ISE over spectrophotometry may be few, because of the unavoidable extraction procedures.

Immunometric measurements of the hormones T_3, T_4 and TSH in serum offer perhaps a more convenient, precise and sensitive approach to estimating thyroid status and hence iodine status. The availability of monoclonal antibodies make immuno-assays more specific and also imply that standardisation of results and increased test efficiency, hence higher throughput, are possible. The main concern is that of cost as there are no local laboratories with the capability to perform the radio-iodination necessary in the assays of particularly T_3 and T_4. By comparison, PBI assays are more cost-effective than immunoassays (Dunn and Haar, 1990). Yet another concern is that of safety in the handling and disposal of radioactive reagents used in the assay. In addition, the cost of gamma counters is still prohibitive.

Therefore, apart from the requirement for species-specific reagents for animals (Ferguson, 1994), it appears that TSH assays may have the safety advantage over the thyroid hormone assays, as they are not based on measurements of iodine levels that

require the use of radioactive materials. Extensive evaluation of non radio-siotope based TSH ELISAs such as enzyme-based chromogenic tests which are not yet available on the market is necessary.

The type of sample is an important consideration in terms of testing efficiency. In using milk samples, it is important to note that daily changes do occur and hence single measurements may not yield reliable estimates (Andrewartha et al., 1980). There is also need for caution in using milk for iodine evaluation as there is a possibility of contamination of milk with iodine-containing teat dips. Also, lactational stages that are likely to be highly varied in unsynchronised herds are likely to distort interpretation of results (Fish and Swanson, 1983; Franke et al., 1983).

In addition, values may be expected to vary with species for instance, while ewes lose 10-50% of the dietary requirements through milk, cows lose only 3-6% (Mason, 1976). Therefore, simultaneous inclusion of standard reference samples nust be considered during screening.

Serum and blood have the advantage that they apply to animals of all ages, sex, breed and types. They can also be collected with minimum damage and time, resulting in measurements that are easier in generalising about, as long as major factors such as temperatures, lactation status and feed contents are known. An additional advantage with blood and serum is the possibility of applying immunoassays such as ELISA and RIA to measure T_3, T_4 or TSH which make it possible to run several samples simultaneously.

While iodine assays can be undertaken on both serum and blood plasma, such measurements are expected to be more tedious and expensive unless the equipment are automated. It is also noted that in situations where imbalances are due to genetic causes, analysis of iodine is unlikely to yield useful information. In such cases, hormonal analysis is advantageous. Serum does not require pre-treatment or digestion for immunoassays and therefore error due to volatilisation or adsorption from such sources is eliminated.

Urine which has been used successfully in human patients, especially as it can be stored without refrigeration (Dunn and Haar, 1990), presents a collection problem as well as possible effects of hydration status dependant on water supply variations and diuresis in individual animal samples.

A variety of laboratory-based confirmatory methods for iodine status are therefore available. Quantitative measurements are possible on samples such as milk, serum, plasma and urine. Of value are measurements of elemental iodine using the ISE method, Gas Chromatography for Thyroxine (T_3 and T_4) and immunometric methods for T_3, T_4 and TSH. These can be supported by evidence of gravimetric histopathological and molecular biologic techniques obtained either before or after laboratory studies. Epidemiological studies using milk and serum could help in routine monitoring and for advisory purposes.

This literature review has described some of the important methods, both direct and indirect, for the determination of iodine status in animals and therefore provides information to enable comparative evaluation of methods appropriate for use in the

estimation of thyroid function in livestock. Methods utilising serum, urine, blood or milk would appear to offer obvious advantages in assessing farm animals namely, cattle, sheep and goats, at herd level.

Generally, assay methods rely on pre-treatment of samples, followed by either chemical determination using titration, spectrophotometry, radioactivity determination, electrolytic determination or chromatography. Thyroid hormone levels can also be determined by various immunological assays among which radioimmunoassays are presently the most important. The increasing move away from use of radio-isotopes is prompting the use of enzyme linked immunoassays mainly for safety reasons.

This review therefore demonstrates that while the importance of iodine is known world wide, information on its impact on livestock raised under natural range conditions such as that prevailing in the Zimbabwean communal and other smallholder production systems where supplementation is rarely used, is largely undocumented. Secondly, routine laboratory diagnostic methods need to be established. A case is made for a study to achieve these two aims as a contribution to improved livestock production.