CHAPTER V

EXPERIMENTAL STUDY ON THE EFFECT OF IODINE SUPPLEMENTATION ON THE PERFORMANCE OF YOUNG CATTLE AT THE MAZOWE FIELD STATION

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AIM

To study the impacts of iodine supplementation on the growth and levels of serum FT_4 in weaner cattle under controlled conditions.

MATERIALS AND METHODS

Trial animals

A convenience sample of station weaner cattle aged between 2 and 15 months was recruited to form the study group. The ages were determined from the farm records relating to each calf by tag and dam identification. The animals were drawn from a herd of station cattle comprising Tuli and Brahman crosses, and from a Fresian dairy herd. Recruitment started with 12 calves in January and ended in March. This allowed the experimental group to increase to the maximum possible of 18, following death of one calf in January and another in February prior to the beginning of the experiment. The Mazowe herd was a closed herd for reasons of disease control.

The calves were divided into two groups, namely control and experimental, using a formal random technique effected by assigning numbers in sequence and then using a random number table for the first and second digits. Even numbers were assigned to the treatment group, and the odd ones to the controls. Nine of the calves were in the experimental group and 9 were in the control group. Each group was housed separately in the quarantine building which is subdivided into two sections. The yards outside the two compartments were separated by a mesh wire fence. Similarly, the paddocks near this quarantine building where the animals spent the day grazing were fenced to ensure that when treatment began in the experimental group, there was no contact with the control group.

All animals were maintained for a maximum of 40 weeks during which health parameters, for example weight gains, temperatures and worm burdens were checked. Iodine supplementation was not given to the treatment group during the first 16 weeks. Therefore all data collected from both groups before week 17 were treated as background ("self-control") data. Apart from grazing predominantly star grass pasture, each animal received a kilogram per day of on-farm snap-corn. During the 24th week of the study, symptoms suggestive of protein deficiency were observed. Thus, a decision to supplement protein using cotton seed cake at 0.5kg per animal per day was made up to the end of the study.

Determination of treatment regimen

Potassium iodate, the source of iodine supplement, was obtained from Milborrows Animal Health$^{®}$ Zimbabwe (Pvt) Ltd. Its purity was not given, but through a titration at

the Toxicology Unit of the Central Veterinary Laboratories, Department of Veterinary Services in Harare, in April 1998, this was calculated to be 83.6%. The supplementation rate was calculated on the basis of information by Olson et al., (1984), that cattle need a total daily dose of 25-30 mg in feed to maintain a euthyroid status (APPENDIX II). Olson et al., (1984) indicated that dietary iodine levels higher than 50mg/animal/day could lead to toxicity. Levels of between 2 and 10mg/kg which Hillman and Curtis (1980), give as toxic thresholds for calves are also far higher than the 25-30 mg total dose for animals of between 50 and 200 kg body weight, such as those used in this experiment.

Preparation and administration of the iodate solution

To obtain an iodate solution that would deliver a total dose of 30mg, 6.0519 grams of potassium iodate per litre of water were required. Determination of this concentration took into account the molecular availability of iodine in potassium iodate, as well as the predetermined level of purity of 83.6%. It was also considered that a volume of at least 10ml of solution would be ideal for ease of oral administration in calves. Therefore, the solution had a concentration of 3mg/ml.

From week 17, each animal in the experimental group was given 10ml of the iodate solution orally every second day. The control group was not given the iodate solution but all other management practices remained the same during the study period for both groups.

Weight measurements and blood sampling

The calves in the experimental and control groups were bled once every two weeks up to the 40th week. The blood samples were kept at 4^0C and allowed to clot. Serum was separated from each blood sample at the Central Veterinary Laboratories, in Harare and aliquoted into 3 vials. These were stored at -20^0C until required for FT_3 and FT_4 analysis using a radio-immuno assay kit method (DPC, 1997).

On each sampling day, and immediately following blood collection, each animal was weighed using a Kettleway$^{®}$ scale that gave the body weights in kilograms.

Data management and analysis.

Data were entered, stored, screened and corrected using Microsoft Office Excel (1997). Descriptive statistics were computed using SPSS version 8 for Microsoft Windows (1998), using the analysis of covariance (ANCOVA) model to investigate the effects of treatment (Daniel, 1983). A mixed effects model with treatment and time as fixed effects, animal as random factor, weight and age as covariates, were fitted. A covariate being a continuous variable that is correlated with the dependent variable, has the effect of improving the accuracy of statistical analysis by adjusting the value of the dependent variable, in this case being FT_4, thus reducing statistical noise. Such an adjustment would be expected to reduce the random variation in the responses and hence provide a more valid estimate of treatment effects. The time factor had 21 levels and treatment had 2 levels. Different time intervals within groups were further investigated using the paired t-test. Differences between groups at particular time

intervals were also investigated using the independent t-test, after checking the assumption of homogeneity of variance. A p-value of < 0.05 was considered significant.

RESULTS.

Descriptive statistics of central tendency are presented in Table 5.1.

Further to this, an analysis of variance showed that the main effects of animal and time were highly significant at $p<0.01$. The covariates weight and age, were also significant ($p<0.03$ and 0.01 respectively). Iodide supplementation was not significant (p-value >0.05), neither was the slight rise in hormone level following protein supplementation after week 26 (Table 5.1).

Table 5.1: Mazowe Station Iodide supplementation experiment: Serum FT$_4$ means and medians (ng/dl) in treatment and control groups, 1998.

Time in weeks	Treatment Group			Control Group		
	Number of observations	Mean serum FT$_4$ values (ng/dl) $\pm$ standard deviation	Median FT$_4$ (ng/dl)	Number of observations	Mean serum FT$_4$ (ng/dl) $\pm$standard deviation	Median FT$_4$ (ng/dl)
1-16	72	0.49 ± 0.251	0.473	119	0.50 ± 0.331	0.474
18-26	45	0.49 ± 0.317	0.449			
28-40	63	0.57 ± 0.322	0.451	52	0.54 ± 0.366	0.474

All pairwise comparisons of control versus experimental group were not significant (p>0.05).

The effect of iodide treatment introduced at week 17 to experimental animals peaked at weeks 17 to 19; and 24 to 32, where the treatment group registered slightly higher means than the control group. A slight rise between 26th week and 32nd corresponded to supplementary feed that was introduced to both groups at that time (Fig 5.1) till the end of the study. Protein supplementation appeared to raise hormone levels marginally, and although not significantly, to a slightly higher level in the treated group.
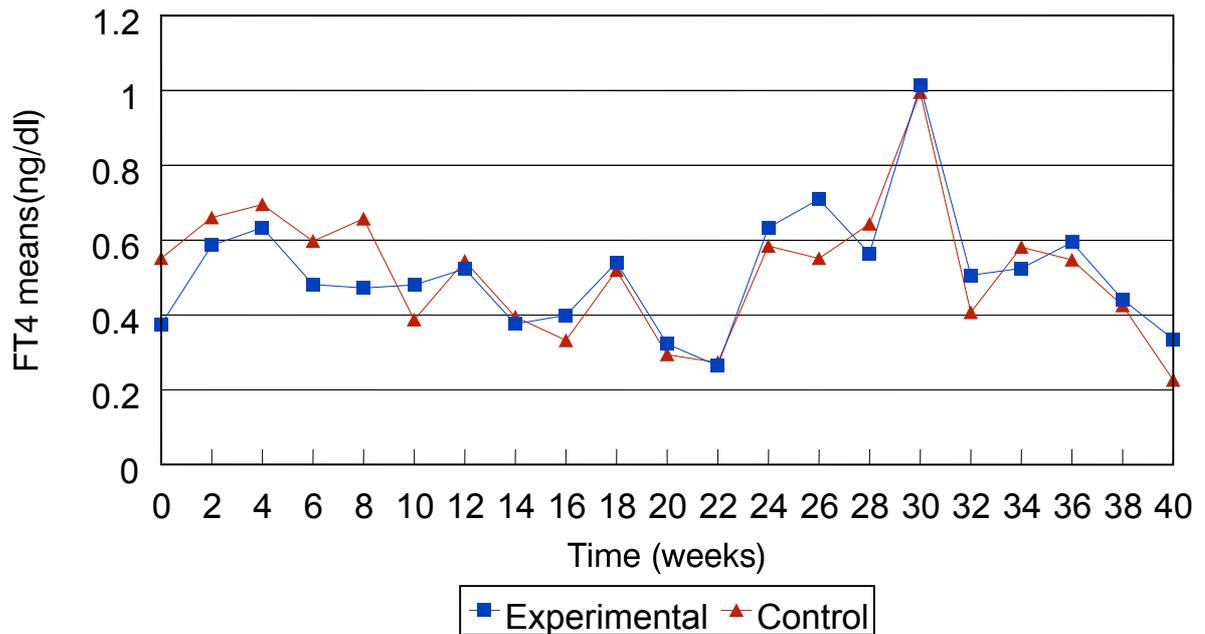
**Fig 5.1.Trends of weekly Serum FT$_4$ means during the Mazowe Station Iodide supplementation study, 1998.**

The pattern of FT$_4$ levels did not indicate an effect due to treatment, but the trend could be consistent with season as they changed from January (weeks 0-4) to October (38-40), and the effect of the protein supplementation causing a peak at week 26. (Fig 5.1)

At the beginning of the experiment, the FT$_4$ levels in the two groups were not significantly different (p>0.05).

Following the iodate treatment, the experimental group's FT$_4$ levels did not vary much from those of the control. From week 24, with the start of protein supplementation, FT$_4$ in the treatment group was slightly although not significantly higher than in the control group, peaking at week 26, while the control group was

noticeably lower. A significant peak occurred in both groups at week 30. This rise was however not sustained.

In contrast to the control group (Fig 5.3), the correlation between FT_3 and FT_4 in the experimental group was almost a straight line with a very weak and non-significant slope ($r = -0.061$ and $p = 0.663$, and $y = -0.278x + 1.984$ (Fig 5.2)). In this relationship, FT_3 therefore remained constant for all levels of FT_4.

r=-0.061   p=0.663
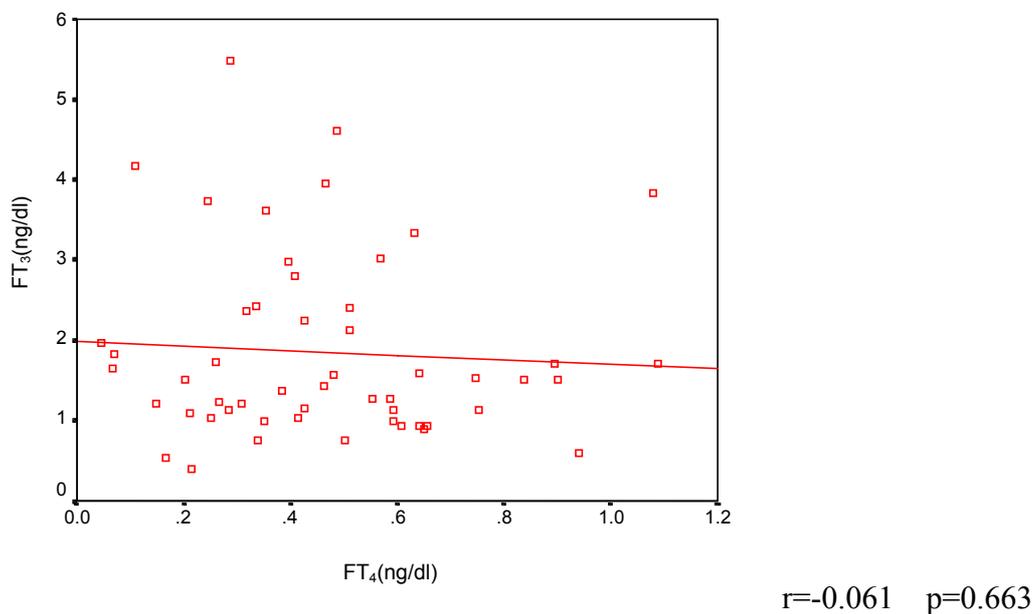
Fig 5.2: Co-relationship between serum FT_3 and FT_4 levels in the experimental group during the Mazowe Station Iodide supplementation study, 1998.

The correlation between FT_3 and FT_4 in the control group was significantly positive with $r = 0.522$ and $p = 0.01$. The correlation equation was $y = 0.209x + 0.216$. The correlation coefficient was positive (Figure 5.3) indicating a rise in FT_3 as FT_4 increases.
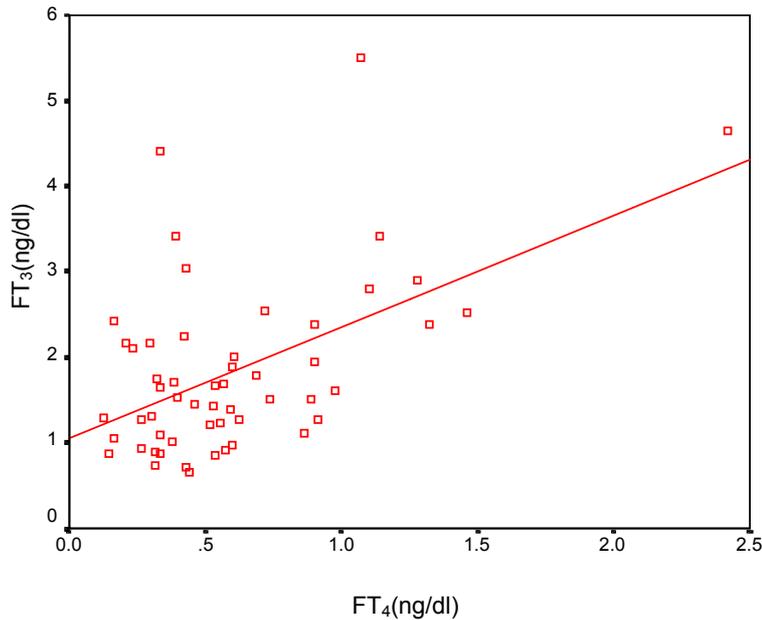
$r=0.522$ $p=0.01$

Fig 5.3: Co-relationship between serum FT$_4$ and FT$_3$ levels in the control group during the Mazowe Station Iodide supplementation study, 1998.

In both groups, weight gains rose more markedly from week 32 (Figs 5.4 a and b), accompanied by a sharp fall in FT$_4$ levels. There were no significant differences in weight between the two groups despite indication in Fig 5.4 a and b showing a slight difference in the first weeks.
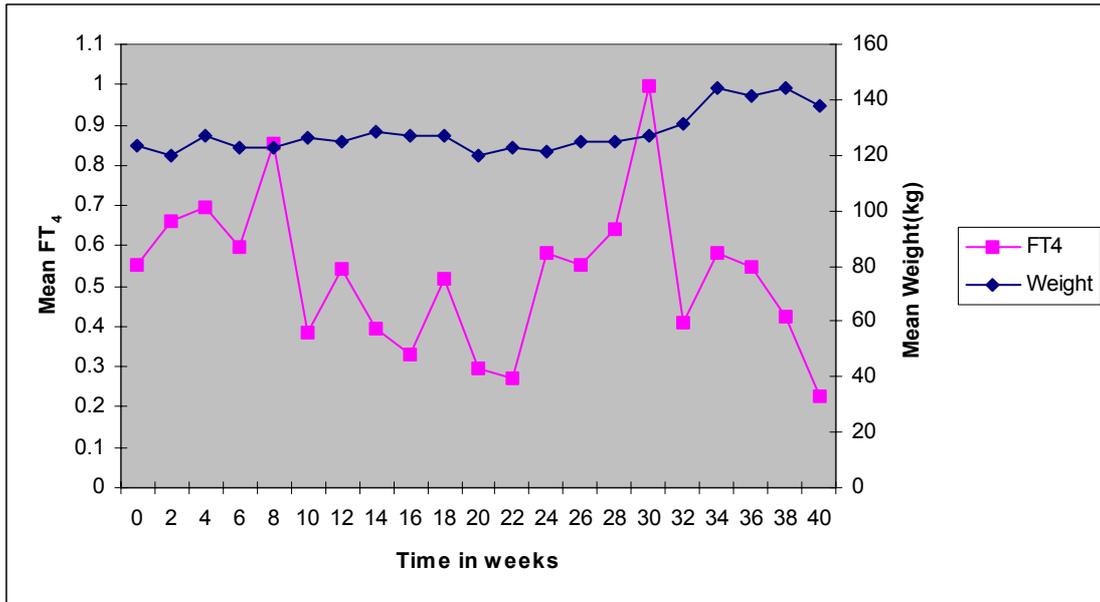
Fig 5.4 a: Patterns of weights and FT$_4$ in control calves group during the Mazowe Station Iodide supplementation study, 1998.
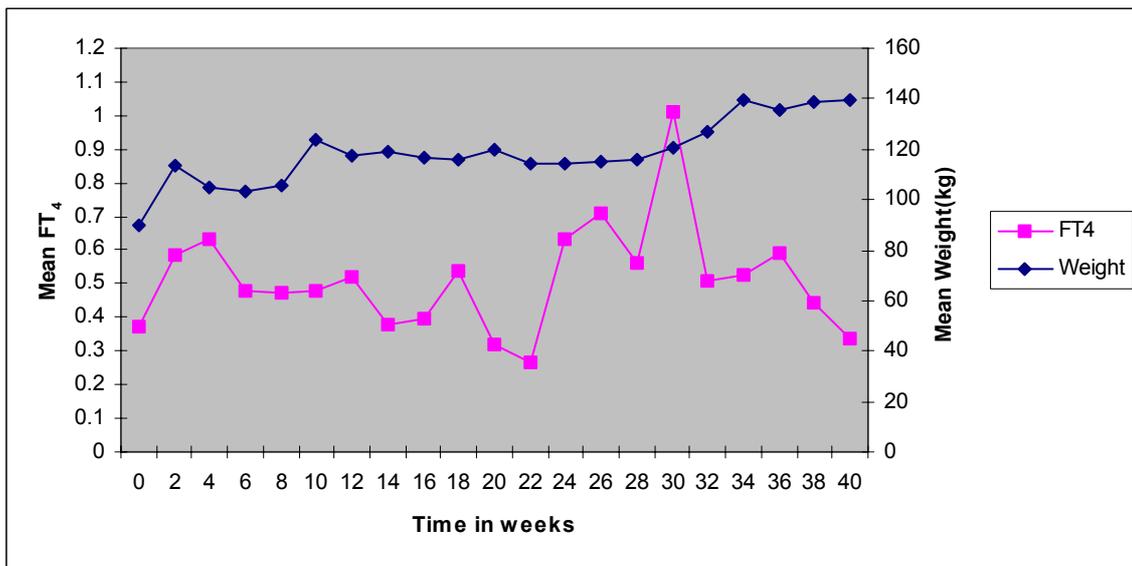
Fig. 5.4b: Patterns of weights and FT$_4$ in experimental calves group during the Mazowe Station Iodide supplementation study, 1998.

FT$_4$ increased significantly with age (months) in the experimental group, with r = 0.237, p=0.001 and y = 0.2003x + 0.300. There was no indication of a fall in FT$_4$

level at 13th month of age (Figure 5.5) at which stage puberty is expected to start setting in.
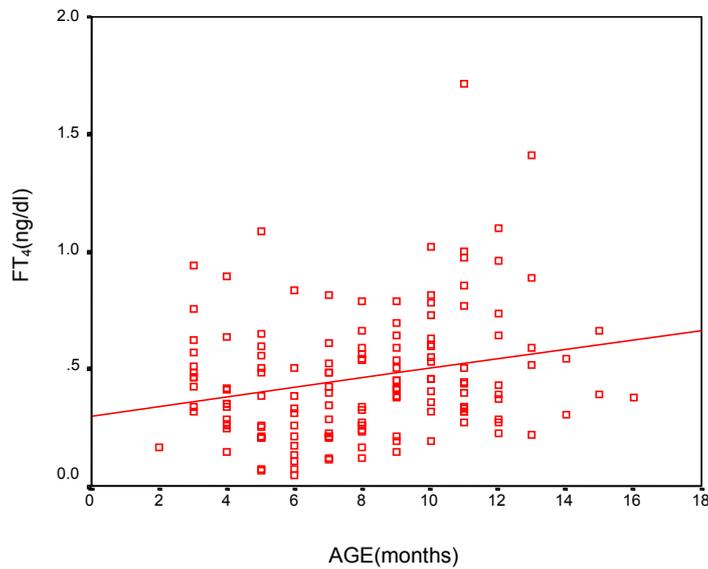
Fig 5.5: Scatter plot of FT$_4$ levels against age in the experimental group during the Mazowe Station Iodide supplementation study, 1998.

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FT$_4$ also had a positive correlation with age in the control group, which remained so at 20 months of age (Fig 5.6).

The slope was slightly steeper in this control group with r = 0.374, p = 0.01 and y = 0.03355x + 0.172 (Fig. 5.6).
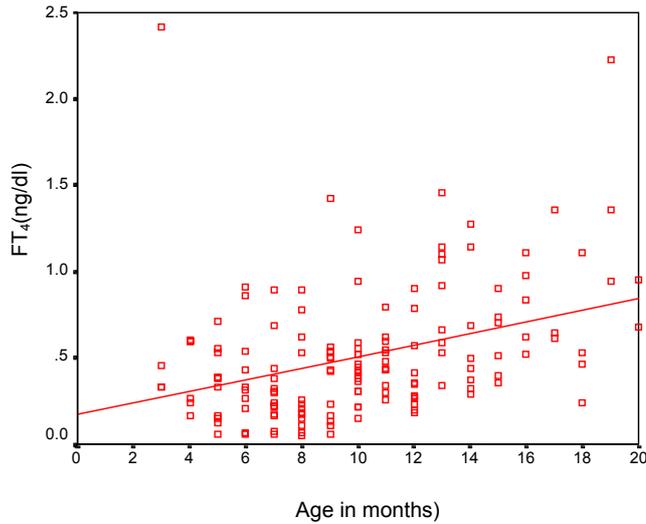
Fig 5.6: **Scatter plot of FT₄ levels against age in the control group during the Mazowe Station Iodide supplementation study, 1998.**

DISCUSSION

Most animals recruited into the study were in the process of being weaned from suckling and ranged in ages between 2 and 11 months. Young animals are known to have higher levels of thyroid hormones than older animals, but in holstein calves, a positive correlation has been reported to exist between T_3 rather than T_4 and body weight up to at least 22 weeks of age (Kahl and Bitman, 1983). In this study (Figure 5.3), a positive correlation between FT_3 and FT_4 in the control group was evident which for FT_3 is in agreement with the findings of Kahl and Bitman (1983) in connection with growth in calves. The constant to slightly negative relationship in the

experimental group (Fig 5.2), suggests that supplementation leads to accumulation of FT_4 even though FT_3 does not rise. This does not, however, explain why in Chapter III, cattle exposed to higher environmental iodine in natural region 4 had lower FT_4 levels, unless it implies a different response in older cattle. It is noted however that these FT_4 changes in the present experiment, were not significant in terms of treatment within the specified time frame.

 Age was a significant factor in the variation of FT_4 levels, and so was weight. Measurements in this study indicate that hormone levels were still rising by the 20th month of age (Fig 5.6).   However. the relationship was slightly more marked (although not significantly so) in the control group (Fig 5.4a). Nevertheless, the rise might have been due to other factors, such as closer health monitoring and a managed feeding regime, or even an increase in reserve of T_4 post- weaning. This situation mimics the finding in Chapter III that where environmental iodine is low, FT_4 levels are high. The relationship of FT_4 and age in the experimental group was slightly less marked, $r = 0.237$ than in the control group, $r = 0.374$ (Fig 5.5 and 5.6), and this is indicative of alterations at metabolic levels due to treatment, similar to the effect of higher iodine exposure levels in natural regions 3 and 4 (Chapter IV), relative to regions 2b and 3 where pasture iodine levels were lower.

Testing 3 types of RIA kits in sheep and cattle sera, Millar and Albyt (1985) found the correlation of FT_4 and FT_3 as well as T_4 and T_3 to be positive with a strong correlation coefficient of greater than 60%. Supplementation with iodide in the present study altered the direction of the relationship between FT_4 and FT_3. The effect of the supplementation levels used in this study could therefore be similar to findings by Rumsey et al., (1983, 1985a) on the effects of Ronnel, an organophosphorus compound, and propylthiouracil respectively in beef steers. They explain a negative

relationship between FT_4 and FT_3 as being due to suppression of deiodination of FT_4 into FT_3, causing a sustained increase in FT_4. A similar relationship is described by Stasilli et al., (1960) in rats fed thiouracil, a known goitrogenic substance, and by Kahl et al., (1978), on beef cattle fed synovex –S, an estradiol/progesterone implant as well as feeding of corn silage alone or in mixture with soybean (Hemken et al., 1965, 1971). Such a situation could in theory lead to slowing down of the breakdown of carbohydrates (reduced calorigenesis) leading to fat accumulation. Although measurements of thyroid binding protein and its uptake rates of total T_4 were not part of this study, comments in the DPC (1991) manual on the COAT-A-COUNT for T_4 suggest that those measurements could possibly have provided more insights on this outcome. It may therefore be concluded that potassium iodate as administered in this study had a metabolic effect on thyroid hormones which did not manifest physically.

Other studies have shown that supplementation of iodide at 1250mg/animal/day may lower T_4 basal concentrations and weight gains of growing Holstein heifers due perhaps to reduced feed consumption and feed efficiency (Leung et al., 1980). According to these authors, cattle are however not as susceptible to thyrotoxicosis as humans, but exceeding 68mg/animal/day has been known to lead to toxicosis. Other studies have indicated that excess dietary iodide may even lead to goitre (Wallace, 1975). As supplementation levels in this study were well below these levels, it could be postulated that the environmental levels at this study site were high enough not to warrant supplementation. It could also be that iodine supplemented in an organic form might be more effective, than in inorganic form as provided in this study (Miller and Swanson, 1973).

Normal levels are attained at dietary iodide levels of 0.2 to 2mg/kg dry matter (DM) or 2.7 to 27mg/animal/day (NRC, 1984) with higher levels required in the presence of goitrogens (ARC, 1980).   The present experiment did not exceed these levels. However, the prior status of the animals had not been defined as deficient either. Indeed, findings in Chapter IV indicated that the general levels in pasture are low. This station is in natural region 2a, which like natural region 4 in that Chapter had higher levels in pasture iodine than other regions (2b, 3 and 5). It is therefore likely that the animals were euthyrodic and did not need more iodine.   However, a study of normal thyroid parameters in 16 North American vertebrate species by Reffetoff et al., (1970), found FT_4 values of 2.5 ng/dl in cattle. Cattle FT_4 values of Mazowe at 0.50 ng/dl are therefore much lower than the normal values in American cattle. Assuming Zimbabwean data found in this study are representative, it would appear that variations by geographical regions can be expected and it might be difficult to make generalisations by comparing values obtained in different regions of the world. It is therefore possible that although values for Zimbabwean cattle are lower, they are euthyroidic. The American values were however measured indirectly using the competitive protein binding assay (Murphy and Jachan, 1965) to determine the total serum T_4 and the percent dialisable fraction. The dialisable fraction described by Oppenheimer et al., (1963) represents the FT_4 levels. The differences noted could well be due to the methods used. Immunoassays are however, considered more refined and sensitive than the earlier competitive protein binding assays (DPC, COAT-A-COUNT T_4,1991).

The purpose of this study was partly to determine the reference levels of FT_4 and factors associated with them since no local values were known *apriori*. It can therefore be assumed that FT_4 levels in young cattle at the Mazowe Veterinary Field Station are 0.5 ±

0.33 ng/dl with a median of 0.47, ng/dl which were the baseline values prior to the commencement of the experimentation.

In this study, iodate supplementation at the rates applied appeared to be ineffective. Except for the change iin the direction of correlation between FT_4 and FT_3, relative to the control group, there were no other clinical findings, indicating differences between the experiment and control groups. This change due to supplementation needs to be studied further, checking the effect of time and age, in a treatment trial. The present experiment only examined a sub-sample for both hormones, without taking time into account. It is therefore difficult to ascribe anything to this supplementation, as differences between the treatment and control groups on weight gain as a measure of growth were very marginal and not significant.

Although the evidence is not strong, protein deficiency especially Thyroid Binding Globulin, could exercerbate a thyroid hormone deficiency if it existed. The observation of poor body condition at about week 24 which showed a depressed FT_4 level in the control group may have been associated with hypoproteinemia as protein supplementation raised hormone levels. If this is true then it further emphasizes the need for holistic approaches in the management of nutritional deficiencies. On the other hand, the observation could be due to ineffectiveness of iodate supplementation on its own, despite the fact that many forms of iodide are said to be effective in correcting hypothyroidism (Ammerman and Miller, 1972; Rudert and O'Donovan, 1974).

Iodide treatment did not result in any significant body weight changes in the experimental group. If however, as argued above, the iodine supplementation levels

were toxic, an effect on performance by weight would be expected. This was not evident clinically. The increase in FT_4 from weeks 1 to 4 may have been due to green pasture since this was in January, a warm wet summer month when vegetative growth of forage is at its highest. This is in comparison with relatively lower levels in June (week 22) and October (week 40).

Assuming that the calves in the experimental group had been euthyroidic prior to iodate treatment, it would appear that the supplementation with iodide may have been beneficial in stabilising FT_4 reserves but, had no influence on weight gains. The treatment could be projected to have long-term effects on serum hormone levels since they changed the direction of the relationship with FT_3. The significance of this observation requires further long-term prospective investigation. Feeding iodide at a rate of 30mg/animal/2 days to the experimental group also needs investigation of longer duration than applied in this study. It is also necessary to test the impact of organic forms of iodine supplements in well designed comparative studies.

CONCLUSIONS

The Mazowe experimental station lies in natural region 2a , which had iodine levels not significantly different from those recorded highest in region 4 (Chapter IV). These levels were however not significantly different from the rest of the natural regions which were much lower. Inspite of this, a supplementation trial under controlled conditions

failed to achieve significant improvement in weight gain of growing calves. Supplementation of weaned calves with an inorganic form of iodine at a rate of about 30mg per animal per day was not effective at this station except that it appeared to alter the direction of the relationship between FT_4 and FT_3. It is likely that this supplementation led to an accumulation of FT_4 reserve relative to FT_3, as occurs when goitrogens are fed.