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To the Graduate Council:

I am submitting herewith a thesis written by Dennis B. Longmire entitled "Effect of Dietary Iodine on Estimated Thyroid Secretion Rates of Cows During Lactation." I recommend that it be accepted for nine quarter hours of credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Dairying.

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EFFECT OF DIETARY IODINE ON ESTIMATED THYROID SECRETION RATES OF COWS DURING LACTATION

A Thesis

Presented to

the Graduate Council of

The University of Tennessee

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

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by

Dennis B. Longmire

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ABSTRACT

Twelve Holstein cows were divided during their first month of lactation into two groups: (1) a control group consuming 4.4 mg per day iodine, and (2) a supplemented group fed an additional 100 mg of iodine per cow daily. All were fed 2.3 kg hay per day, corn silage ad libitum, and averaged 13.4 kg per day of 18% crude protein concentrate containing 1% common NaCl.

Production of milk, fat, total solids, and consumption of feed during the first 26 weeks of lactation were not significantly different between groups. Plasma protein-bound iodine (PBI) and thyroxine secretion rate (TSR) were determined four times. Mean plasma protein-bound iodine was 3.81 µg per 100 ml for Group 1 and 6.96 for Group 2 ($P < .05$). Mean thyroxine secretion, estimated from iodine utilization rate, was 1.96 mg per day for Group 1 and 2.53 for Group 2 ($P < .05$). Last period plasma protein-bound iodine determinations averaged 84 and 59% thyroxine for the two groups. Thus, actual thyroxine secretion rates for the groups were about equal. Milk iodine of Group 1 averaged 28.9 µg per liter compared to 319.6 for Group 2.

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CHAPTER I

INTRODUCTION

To support maximum milk production in the dairy cow a proper complement of metabolism regulating hormones must be present. Thyroxine is essential to the complex controlling the energy metabolism rate as well as being galactopoietic in the cow. Iodine is the major dietary requirement of the bovine for thyroxine formation. A deficiency of iodine causes the thyroid gland to enlarge in an effort to obtain the iodine needed from the blood. Iodine is lost from the body via the gut, kidneys, and mammary glands of the lactating cow.

The possibility exists that sufficient iodine may be secreted in the milk at peak lactation to result in an imbalance between iodine intake and excretion. Such an imbalance would be more likely to occur in high producing cows. The requirements for iodine of cattle have not been clearly defined, nor have the effects of a deficiency on lactation. If such a deficiency limits the thyroid secretion rate of hormones it could result in subnormal milk yields.

This investigation was conducted to determine the effects of iodine supplementation of a low iodine diet on the thyroid secretion rate and milk yield of high producing cows.

CHAPTER II

REVIEW OF LITERATURE

Mechanics of Iodine Metabolism

Iodine has long been known to be a necessary dietary element for the prevention of goiter. The prevention of goiter in the newborn animal was the basis for several early studies of iodine in this country. Hart and Steenbock (23) used potassium iodide in the ration of pregnant sows to prevent hairless and goitrous pigs. The goitrous symptoms and prevention are well accepted, but other glands possess mechanisms for iodine uptake, utilization, and excretion in addition to the thyroid (10).

Inorganic iodine is absorbed approximately 100% from the gut and most of the fecal iodine is organic in nature (4, 24). Normally organic iodine is conjugated in the liver and secreted into the bile. Much of the organic iodine is reabsorbed but fecal loss can be substantial.

In mammals, the main extrathyroidal concentrating mechanisms are the salivary gland, the stomach, the skin, and the mammary gland, placenta, cervix, and kidney (10). The ability to concentrate iodine in these various glands varies from species to species. In the bovine it appears that the kidney, stomach, and mammary gland are the main extrathyroidal pathways of excretion.

Archibald (5) found milk levels from 0.01 to 0.70 ppm for non-supplemented cows with a mean of .043 ppm (43 μg/liter). Hanford (22) reported a study involving three groups: controls, low iodine

supplementation (50 mg/day) and high iodine supplementation (250 mg/day). The level varied with grain intake as it was fed as a grain premix. The controls had an output in the milk of 0.345 mg/day or 11.2% of the total intake; the low level group had an output in milk of 5.65 mg/day or 12.29% of the intake; and the high level group excreted 23.63 mg/day in the milk or 11.9%. The production levels of the cows were not reported. Matthews et al. (44) reported a mean of 47 mg/day excreted in the milk of cows yielding 21 liters/day or 25.3% of the intake (186 mg/day) lost to the milk; whereas with cows yielding 50 liters/day there was 5.4 mg/day found in the milk, or 5.7% of the 94 mg/day intake. Flamboe and Reineke (16) also reported significantly lower iodine concentrations in milk during the summer than the winter months. They reported a milk concentration of I^{131} approximately six times that of blood concentration 50 hours after dosing. During the first 50 hours after I^{131} administration, an average of 26.7% of the dose was secreted in the milk and only 9.6% retained by the thyroid. They interpreted these data as indicating that, by the udder-kidney-thyroid competition for available iodine, a functional iodine deficiency could be created, resulting in a reduced thyroid secretion rate. This was substantiated by a negative correlation ($P<0.01$) between milk yield and thyroid secretion and also between milk yield and I^{131} uptake. Miller and Swanson (45) have shown that iodide passes easily in both directions between mammary gland and blood; however protein-bound iodine (PBI) did not appear to transfer in either direction. It was concluded that iodide enters the udder passively, but once in the milk collecting spaces, a portion is bound to milk protein and not available for reabsorption;

consequently the more milk secreted, the greater the binding power, and the more iodine excreted via the mammary gland.

Miller et al. (46) found seven-day I^{131} excretions from oral doses of NaI^{131} to average 4.0% in milk, 38.8% in urine and 23.7% in feces. Lengemann (34) reported excretions of 5.8, 47 and 15% in the milk, urine and feces, respectively, in cows given I^{131} orally and receiving approximately 3.5 mg iodine/day. With cows receiving 12-16 mg iodine/day he recovered 7.5, 66, and 16% in the milk, urine and feces, respectively.

The most physiologically important iodine concentration mechanism is the thyroid gland. The thyroid functions in several several phases in the formation and secretion of thyroid hormone: (1) "trapping" of inorganic iodide from circulating blood; (2) concentration of inorganic iodide in the gland; (3) synthesis of thyroglobulin in follicular cells; (4) storage of thyroglobulin in colloid; (5) liberation of thyroxine and triiodothyronine from thyroglobulin; and (6) passage of these compounds into general circulation (12). These functions of the thyroid are mainly under the control of pituitary thyroid stimulating hormone (TSH). Release of TSH is mediated through the hypothalmus which is under negative feedback control of circulating thyroid hormone (60). There is evidence in human clinical practice that the feeding of massive doses of iodine (100 times the normal intake) can be beneficial in treating hyperthyroidism, probably through the inhibitory effect of non-thyroidal protein-bound iodine on the pituitary, causing a decrease in thyroid stimulation (60, 14).

Iodine Requirements for Lactating Cows

The National Research Council (51) recommendation for dairy cattle suggests the use of iodized salt containing .0076% iodine incorporated at a 1% level in the grain mix. This is based mainly on a report by Griem et al. (19) and the studies of Kalkus (32). Griem suggested that stabilized iodide in salt will provide 3.5 mg of iodine per day if 4.54 kg of grain is fed. The 3.5 mg of iodine would supply three and one-half the calculated requirement. Griem (19) based his requirement, assuming a 500 kg cow, upon calculations from estimates of the human requirement, but he ignored the loss of iodine in milk. All reports cited give an iodized salt level in the feed with no actual quantitative data as to requirement.

The British Agricultural Research Council (9) requirement is somewhat higher than the NRC recommendations, assuming that iodized salt is the only source of iodine in some geographical areas. The ARC recommendation is for 0,80 mg I per kg dry matter for pregnant or lactating ruminants and 1.20 mg/kg dry matter if feeds contain goitrogens. The NRC recommendation on a comparable basis would be 0.76 mg/kg grain or about one-half the British recommendation if the cow received one-half of her intake in the grain.

Factors Affecting Iodine Uptake

There are limited analyses of feed samples for iodine content and these are extremely variable. Alderman and Jones (2) reported a reduction in iodine content of forages with increased nitrogen fertilization. They also found differences in species and harvest

dates, but none to which they assigned significance. The use of nitrogen fertilizers could also be related to the forage nitrate level. Nitrate has been negatively correlated to thyroid function. I^{131} uptake decreased and weight gains decreased in rats with nitrate feeding (8, 13). Sampson and Putzki (59) reported that iodine requirements may be raised on high calcium diets. This has not been further substantiated.

In other work by Alderman and Jones (2), it was found that the iodine content of forages was significantly influenced by date of harvest as well as by species. The iodine content varied from 0.20 to 0.32 ppm in the species studied. The range in iodine values during the season ranged from 0.14 to 0.45 ppm.

The goitrogenic effect of feeding soybean oil meal was first reported by McCarrison (42) in 1934 and later by Sharpless et al. (61) in 1939. Both Sharpless et al. and Halverson et al. (21) reported that heating would reduce the goitrogenic effects. Van Middlesworth (68, 69) and Beck (7) have studied the mode of goitrogenic action of soybean protein and have concluded that soy protein in some way interferes with the normal reabsorption of organic iodine from the gut and does not effect the absorption of inorganic iodine. The increased fecal loss of iodine produces a drain on the iodine pool and can lead to a hypothyroid condition if additional iodine is not furnished in the diet. Hemken et al. (26, 27) have reported that cows receiving large quantities of soybean meal in their ration with no supplemental iodine gave birth to calves with enlarged thyroids. Cows fed cottonseed meal and urea as the protein supplement produced calves

with normal thyroids. Kaunitz and Johnson (33) have reported that animal fats and vegetable oils increase the iodine requirement.

Effects of Iodine Supplementation

The role of possible iodine deficiency on reproductive performance has not been studied under controlled conditions; however, two field studies conducted in iodine deficient areas have shown a possible relationship between iodine supplementation and reproductive performance. McDonald et al. (43) have shown that the conception rate of repeat breeder cows could be improved by treating with organic iodine for 8 to 12 days before the onset of estrus. Moberg (49) reported significantly increased conception rates with cows receiving supplemental iodine from those not receiving iodine. The cows receiving iodine also exhibited significantly fewer irregular breeding intervals as well as fewer retained placentas. Hemken (25) has also reported a higher incidence of retained placentas in apparently iodine deficient cows.

Moberg (49) reported a significant decline in the incidence of ketosis when supplemental iodine was given. This is in accord with Robertson et al. (58), who concluded that ketosis can be the result of adrenal cortex insufficiency, which can be caused by hypothyroidism. Although iodine reduced the incidence of ketosis, it did not eliminate the problem, suggesting that hypothyroidism may increase the susceptibility to ketosis.

Cows consuming a goitrogenic ration have shown an increase in milk production, increased fat percentage, increased forage

consumption, and greater mobilization of body energy stores when iodine was added to the ration (27, 67). The performance of the iodine treated cows in comparison to the untreated cows was similar to the response from thyroprotein feeding.

There are two reports (27, 67) indicating that the feeding of inorganic iodine significantly raised the serum protein-bound iodine (PBI) level; however, these authors postulated that this rise in serum PBI may simply be an artifact of high supplemental iodine and may not have indicated true differences in hormonal iodine. Long et al. (40) found that supplemental potassium iodide as high as 22 mg per day had no effect on the PBI level. This was considered to be twice that amount of iodine likely to be consumed by the recommended feeding of iodized salt.

Estimation of Thyroid Secretion Rate

Thyroxine is considered the major form of circulating thyroid hormone with lesser amounts of triiodothyronine being present (60). The majority of the thyroxine is considered to be protein bound. PBI determinations have been used extensively as an index of thyroid activity in both clinical and experimental situations due to the fact that low PBI values are associated with hypothyroidism and high PBI values with hyperthyroidism. However, PBI values are not completely valid as measurements of thyroid activity in cattle because nonthyroidal iodine compounds have been shown to bind to proteins (18).

The most accurate method, to date, of measuring thyroid gland activity in dairy cattle has been the determination of estimates of

thyroid secretion rate (TSR) and the physiological variations thereof. The first determination made in domestic animals was the thyroxine substitution method whereby the TSR is estimated from the minimum amount of thyroxine administered daily that will block the release of I^{131} previously injected and subsequently trapped by the thyroid. This method was first used by Henneman et al. (28, 29), in sheep. It was confirmed and expanded by Reineke and Singh (57) in rats. Further studies with sheep have been reported by Singh et al. (63). This technique has also been employed in dairy calves by Lewis et al. (27), Pipes et al. (52), and Lodge (39), and in dairy goats by Flamboe and Reineke (16). Ingbar and Freinkel (31) demonstrated that the amount of thyroxine (T_4) degraded by the body each day is approximately equal to that synthesized by the body, or released by the thyroid daily, in a relatively steady physiological status. This allowed the use of thyroxine degradation rates as estimates of thyroid secretion rates. Several investigators have reported success with this method, using injected I^{131} labeled thyroxine to chart the rate of disappearance of T_4. It requires only four days time for the determination. An adaptation of the I^{131} labeled thyroxine turnover method was reported by Mixner and Lennon (48) in which the thyroid secretion rate is determined from the turnover of normal thyroxine, eliminating the need for radioisotopes.

Post and Mixner (54) compared the thyroid secretion rate determinations made by the stable thyroxine turnover method of Mixner and Lennon (48), the radiothyroxine degradation rate method of Freinkel and Lewis (17), the thyroxine replacement method of the

thyroxine inhibition of thyroidal I^{131} release, and their own method of radiothyroxine turnover method based on the isotope dilution procedures of Peterson and Wyngaarden (53). The isotope dilution procedure was used as a standard. The isotope dilution method using radiothyroxine and the chemical thyroxine turnover methods were significantly correlated ($P<0.01$). The thyroxine turnover methods were considered reliable and allowed determinations to be made more rapidly than by other methods. Of these turnover methods, the isotope dilution method was considered most accurate; but where use of radio-isotopes would be undesirable, the chemical thyroxine method was considered to give good results. Vohnut et al. (76) reported that thyroid secretion rate measured by substitution therapy would be higher than thyroxine degradation rate measured by plasma clearance rates since iodine escapes from the thyroid due to deiodination during hydrolysis of thyroproteins. However, degradation rate was considered to be of more physiological significance since it was more directly related to the hormone portion actually available to the cells.

Variations in Thyroid Secretion Rates

Information in the variation in thyroid secretion rates in cattle is limited and somewhat contradictory. Lodge et al. (39) reported lowered rates in summer in heifers, but the same group (37) failed to confirm this in a subsequent report. Swanson et al. (66), using the uptake of I^{131} as the index of thyroid activity, observed little seasonal variation. Premachandra et al. (55) reported a variation between cows in the depression of thyroid secretion rate

during summer months. It was suggested that some animals have the capacity to withstand high temperatures with less reduction in thyroid secretion rate and subsequent milk production.

Grosvenor and Turner (20) reported a significantly higher thyroid secretion rate in rats during the period of peak lactation than in non-lactating rats of the same strain, age, and weight. These authors concluded that a hyperthyroid state is desirable for optimum milk secretion.

Mixner et al. (47) reported protein-bound iodine (PBI) to be lowest immediately after parturition, rising during lactation. Total thyroxine secreted remained fairly constant throughout lactation; however, there was a significant ($P<.01$) drop in thyroxine secretion rate (TSR) per 100 lb body weight. The TSR/day was 1.763, 1.618, 1.619, 1.658 mg/day at 2 weeks, 3 months, 6 months, and 9 months of lactation, respectively. They also reported that blood levels of thyroxine, as measured by PBI, during changing physiological conditions did not correspond to TSR, due to an inverse tendency in the volume of distribution of the thyroxine.

Lorscheider et al. (41) using the Tetrasorb-125 method found serum T_4 to be significantly reduced during peak lactation in cows when compared later in lactation. A subsequent report by Etta and Reineke (15) concluded that during heavy lactation there is a decrease in serum T_4 without a decrease in thyroxine-binding globulin. These workers have postulated that during peak lactation, with limited iodine intake, lactation could become self-limiting as a result of reduced thyroid function due to the mammary-thyroid-kidney competition for iodine.

Summary of Review of Literature

Thyroxine has been shown to be an important galactopoietic hormone. The major dietary requirement of the bovine for the formation of thyroxine is iodine. Iodine is lost from the body in several ways, including the mammary gland--a loss which may be significant under certain conditions. The possiblity of a physiological deficiency in high producing cows exists; as supplemental iodine has been shown to be beneficial under certain situations. The data relative to the effect of this supplemental iodine on the thyroid secretion rate are inconclusive so far as the high producing cow is concerned.

CHAPTER III

EXPERIMENTAL METHODS

This experiment was conducted for 26 weeks in early stages of lactation. Six pairs of 3, 4, and 5-year old Holstein cows were selected from the University of Tennessee dairy herd on the basis of high production in the previous lactation. All cows calved within a 35-day period during November and December, 1969. Each pair was started on the trial within the first month of lactation. The cows were paired soon after calving on the basis of size, age, and current level of milk production. All cows were housed in the same area of the barn in tie stalls.

Feeding Schedule

During the experimental period, all cows were fed an 18% protein grain mix consisting of 50% ground corn grain, 24% ground oats, 25% of a 42% soybean oil meal, and 1% plain salt. All cows were fed at the rate of 1 kg grain mix per 2.5 kg milk. In addition, one cow in each pair was given 10 grams of a sucrose mixture containing 131 mg KI, or 100 mg iodine, which was mixed once daily in the grain mixture. All cows received 2.27 kg of mixed grass-legume hay daily. Silage was fed twice daily. Hay refusals were minimal, so hay consumption was recorded the same as fed. Silage feeding was adjusted to minimize refusals. Refused silage was weighed before each feeding.

Feed Analysis

Samples of the grain mix, silage, and hay were taken at two-month

intervals and analyzed for iodine content, as well as proximate chemical constituents (1, 6). The feed composition is summarized in Table I.

Milk Yields and Composition

Milk weights, as read from calibrated weigh-jars, were recorded at each milking. Weekly milk samples were drawn from the weigh jars from two consecutive milkings on each cow. Milk samples were analyzed for butterfat and total solids composition. The cows' udders were washed at each milking with a commercially mixed quaternary ammonium compound solution to reduce iodine in contact with the udder. The post milking iodine teat dip procedure practiced routinely with the remainder of the herd was not used. Four times during the 26-week experimental period milk samples were taken and analyzed for iodine by the alkaline ashing method (1). Milk samples were obtained after thoroughly washing the udder with a hypochlorite solution. Sampling was limited to fore-milk obtained about two hours before the regular afternoon milking.

Blood Analysis

At six-week intervals 30 ml of blood were drawn from the jugular vein for total iodine and protein-bound iodine (PBI) determinations on two consecutive days. The PBI determinations were carried out according to the method of Brown (11) as modified by Lennon and Mixner (36). Total plasma iodine was determined in a similar method with the total plasma being ashed instead of only the protein precipitate as in the PBI determination. The preliminary blood analysis was made

TABLE I

AVERAGE FEED ANALYSIS OF BASIC RATION[a]

Ingredient	Moisture	Protein	Fat	Crude Fiber	Ash	N.F.E.	Iodine
	----------	----------	%	----------	----------	----------	(µg/kg)
Concentrate	13.01	18.36	4.32	4.52	1.32	58.47	199.9
Corn Silage	63.32	3.27	0.73	6.53	1.27	24.88	82.1
Mixed Hay	9.51	12.55	1.95	27.56	7.26	41.17	116.3

[a]Feed sampled at two month intervals includes four analyses.

immediately before the start of the experimental feeding schedule. Four additional analyses were made during the iodine-feeding period.

Thyroid Secretion Rate Estimations

After blood samples had been taken for analysis as described previously, each cow was injected intravenously with 10 ml of a Na-L-thyroxine solution containing 2 mg of thyroxine per ml of water. According to the method of Post and Mixner (54), blood samples were taken twice daily at 8 or 16 hour intervals for three days to determine the amount of thyroxine being degraded or eliminated from the extrathyroidal pool per unit time. It is assumed that, under homeostatic conditions of constant pool size, the amount of thyroxine degraded must equal that being secreted. In this method, the thyroid secretion rate (TSR) was calculated as the product of the normal preliminary level of blood thyroxine measured as the plasma protein-bound iodine (PBI), the thyroxine fractional turnover rate per day (TFTR), and the volume of distribution of thyroxine (TVD). Extra-thyroidal thryoxine (ETT) can be estimated as the product of PBI (thyroxine) and TVD. Thus, TSR would also be the product of ETT and TFTR. The turnover of normal L-thyroxine is calculated from the decline in total plasma PBI values 16 to 72 hours after the 20 milligram dose of L-thyroxine had been injected as illustrated in Figure 1. Degradation curves were considered to be exponential, as represented by the equation: $Y = a_e^{-bt}$ or, for calculation purposes, the logarithmic form, $\ln Y = \ln a - bt$; whereas Y = total PBI, t = time in hours, a = Y-axis intercept (time 0) and b = regression

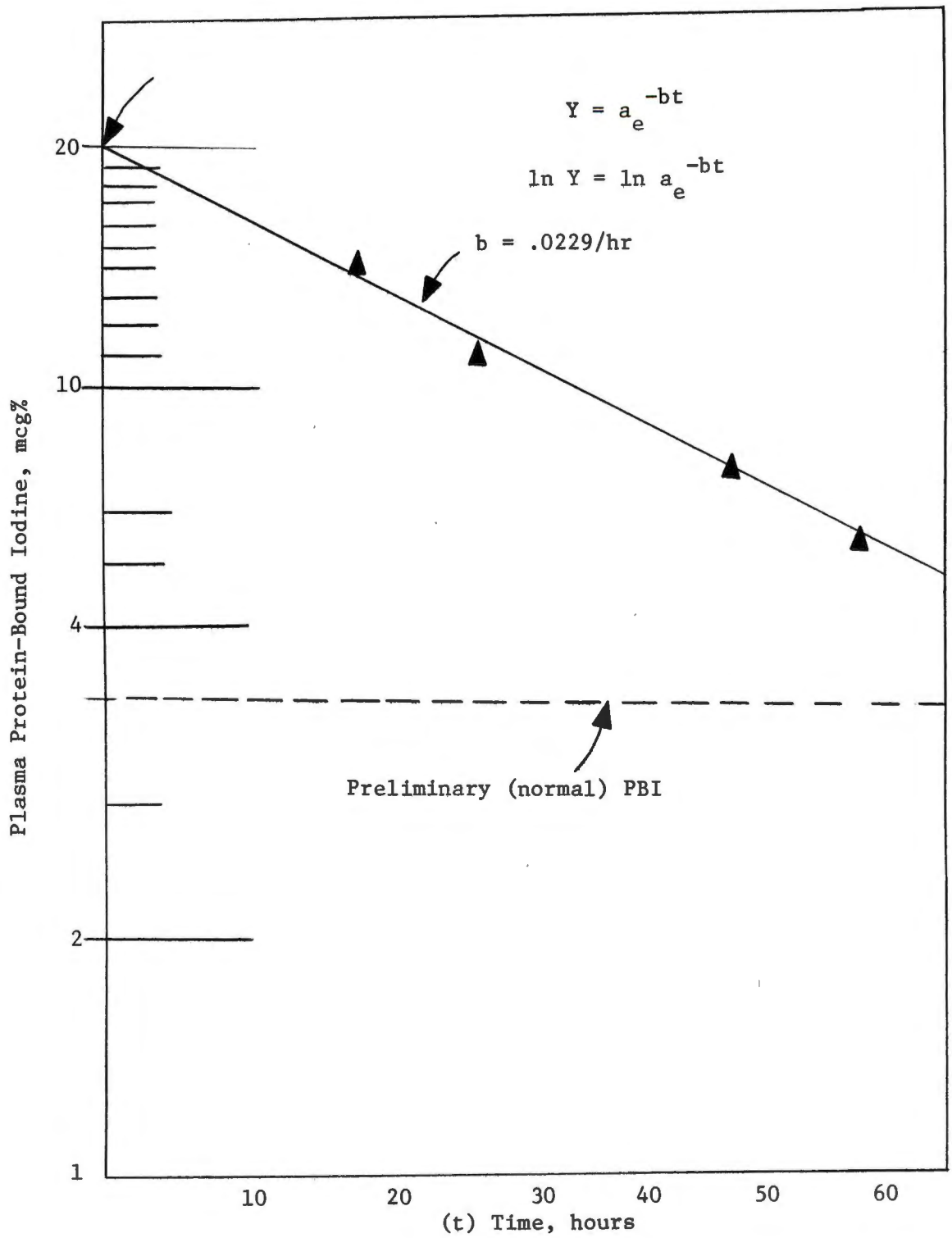


Figure 1. The turnover of a single intravenous injection of 20 mg of L-thyroxine in the cow. Zero is time of injection.

coefficient or thyroxine turnover rate per hour. The thyroxine fractional turnover rate was calculated as the b value in the above regression equation times 24 hours to give the rate per day. The b value was determined by dividing the difference between the natural logarithm of the PBI at any two times by the number of hours between the two points.

The total volume of distribution (TVD) was calculated from the following equation:

$$\text{TVD, liters} = \frac{\text{Dose of thyroxine, 20 mg x 100}}{(a-n)/0.653}$$

Where a (x-axis intercept) equals the theoretical concentration of PBI at zero time, and n is the normal or preliminary PBI level. The constant, 0.653, was used to convert iodine to its equivalent as thyroxine.

Plasma Thyroxine Levels

In an effort to determine if differences in protein-bound iodine caused by iodine treatment were indicative of true plasma thyroxine differences, or were simply artifacts caused by the non-thyroidal PBI, analyses were made using the Tetrasorb-125 test of Abbott Laboratories which is a modification of the competitive protein-binding analysis of Murphy et al. (50). The Tetrasorb test is a competitive-protein-binding method which has been shown to have a higher diagnostic accuracy for plasma thyroxine than PBI values alone (50).

Statistical significance of observations was tested by means of the paired t-test according to Snedecor (65).

CHAPTER IV

RESULTS AND DISCUSSION

Effects on Feed Intake

The silage and grain intake data are given in Table II. The grain intake was influenced by production level. All cows were fed at a constant milk-to-grain ratio with grain fed adjusted as milk production declined. No significant differences ($P>.05$) were found with either silage or grain intakes that could be attributed to the level of iodine intake. The small amount of hay fed to each cow was practically all consumed. The mean daily iodine intakes from the various sources are summarized in Table III. The intakes were essentially equal between groups by feed sources; however, the 100 mg/day addition to the iodine supplemented group resulted in their intake being approximately 25 times that of the control group. There was no apparent difference in the amount or the composition of the feed consumed by the cows in the two groups, except for the amount of iodine ingested.

This lack of intake response differs from the results of Hemken (27) and Lengemann (35) who reported increased forage consumption in cows receiving goitrogens after iodine supplementation. In their reports, all cows had been on iodine deficient rations for one year prior to the start of supplementation. The supplementation was also started during the dry period. The fact that the cows had calved while on a low iodine ration could account for the differences they reported. Also their trials utilized corn silage as the only roughage;

TABLE II

MEAN DAILY FEED INTAKE DURING EXPERIMENTAL PERIOD OF IODINE SUPPLEMENTED AND CONTROL COWS

Iodine Supplemented			Control			Iodine Supplemented Difference	
Cow	Silage	Grain	Cow	Silage	Grain	Silage	Grain
(No.)	(kg)	(kg)	(No.)	(kg)	(kg)	(kg)	(kg)
305	15.18	12.66	410	20.86	14.07	-5.68	-1.41
311	23.99	11.93	402	14.92	10.89	9.07	1.03
321	17.50	15.00	266	23.53	13.62	-6.02	1.38
370	12.74	15.30	315	17.89	15.43	-5.16	-0.13
433	17.50	12.09	339	13.74	12.42	3.76	-0.33
444	19.54	12.95	422	15.26	14.37	4.27	-1.43
Avg.	17.74	13.32		17.70	13.47	0.04	0.15

TABLE III

MEAN DAILY IODINE INTAKE DURING EXPERIMENTAL PERIOD OF IODINE SUPPLEMENTED AND CONTROL COWS

Iodine Supplemented						Control					Iodine Intake
Cow	Silage	Grain	Hay	Supplement	Total	Cow	Silage	Grain	Hay	Total	Difference
(No.)	(mg)	(mg)	(mg)	(mg)	(mg)	(No.)	(mg)	(mg)	(mg)	(mg)	(mg)
305	1.25	2.53	0.26	100.0	104.04	410	1.71	2.81	0.26	4.78	99.26
311	1.97	2.36	0.26	100.0	104.61	402	1.22	2.18	0.26	3.66	100.95
321	1.44	3.00	0.26	100.0	104.70	266	1.93	2.72	0.26	4.91	99.79
370	1.05	3.06	0.26	100.0	104.37	315	1.47	3.08	0.26	4.81	99.56
433	1.44	2.42	0.26	100.0	104.12	339	1.13	2.48	0.26	3.87	100.25
444	1.52	2.59	0.26	100.0	104.37	422	1.25	2.87	0.26	4.38	99.99
Avg.	1.45	2.66	0.26	100.0	104.37		1.45	2.69	0.26	4.40	99.97

whereas, hay was fed at low levels to all cows during the trial reported here.

Effect on Milk Yield and Composition

The total milk production during the 26-week experimental period, as well as the mean daily yield, are given in Table IV. The iodine supplemented cows produced an average of 3.3 kg per day less than the controls. This difference was not statistically significant ($P>.05$) and could be attributed to random differences between pair mates. It should be noted that, although there was no significant decrease, neither was there an increase in production from iodine supplementation, as had been observed by Hemken (27).

The milk fat and total solids production is summarized in Table V. As with milk yield, no significant differences were found in either the percentages or total production of milk fat or milk total solids. The iodine intake of the control cows (0.223 mg/kg dry matter) was well below the recommended level of 0.80 mg/kg of dry matter as proposed by the British Agricultural Research Council (9) for lactating cows. In addition, the cows were being fed high levels of both soybean oil meal and corn silage, a ration which has been shown to be goitrogenic (26). Based on these data, the control cows should have been approaching an iodine deficiency condition. However, the possibility exists that the 26-week period was not long enough to deplete the iodine previously stored by the thyroids of these cows. The supplemented cows had an iodine intake of approximately 5.2 mg/kg dry matter which would be over four times that of the British recommendation. Even with these

TABLE IV

MILK PRODUCTION OF IODINE SUPPLEMENTED AND CONTROL COWS

Iodine Supplemented			Control			Iodine Supplemented Difference	
Cow	Mean Daily Yield	26 wk Yield	Cow	Mean Daily Yield	26 wk Yield	Daily	Total
(No.)	(kg)	(kg)	(No.)	(kg)	(kg)	(kg)	(kg)
305	26.8	4875.4	410	32.6	5925.9	-5.8	-1050.5
311	26.1	4754.8	402	30.8	5596.6	-4.7	-841.8
321	34.3	6243.6	266	30.8	5612.9	3.5	630.7
370	33.2	6039.8	315	40.7	7402.7	-7.5	-1362.9
433	22.2	4033.4	339	24.9	4540.5	-2.7	-507.1
444	26.6	4833.6	422	29.0	5280.9	-2.4	-447.3
Avg.	28.2	5130.1		31.5	5726.6	-3.3	-596.6

TABLE V

MEAN DAILY MILK FAT AND TOTAL SOLIDS PRODUCTION OF IODINE SUPPLEMENTED AND CONTROL COWS

Iodine Supplemented					Control					Iodine Supplemented Difference			
Cow	Milk Fat		Total Solids		Cow	Milk Fat		Total Solids		Milk Fat		Total Solids	
(No.)	(%)	(kg)	(%)	(kg)	(No.)	(%)	(kg)	(%)	(kg)	(%)	(kg)	(%)	(kg)
305	3.45	.92	12.03	3.22	410	3.36	1.09	12.96	4.22	0.09	-0.17	-0.93	-1.00
311	3.55	.93	12.14	3.17	402	2.46	.75	9.89	3.04	1.19	0.18	2.25	0.13
321	3.49	1.20	12.28	4.21	266	3.67	1.13	11.97	3.69	-0.18	0.07	0.31	0.52
370	3.06	1.01	11.29	3.75	315	3.43	1.39	11.94	4.86	-0.37	-0.38	-0.65	-1.11
433	4.48	.99	13.10	2.90	339	3.63	.91	12.49	3.12	0.85	0.08	0.61	-0.22
444	3.31	.88	11.61	3.08	422	3.22	.93	13.31	3.86	0.09	-0.05	-1.70	-0.78
Avg.	3.56	.99	12.08	3.39		3.29	1.03	12.09	3.80	.28	-0.04	-.01	-0.41

widely different iodine intakes, no differences were measured in lactation performance of the two groups. No differences ($P>.05$) were obtaine in lactation persistency between the two groups. No deficiency symptoms were observed in cows on the low iodine ration.

There was, however, a significant difference ($P<.01$) in both the concentration and amount of iodine secreted in the milk from the two groups. As shown in Table VI, the supplemented cows excreted from 6 to 19 times as much iodine through the mammary gland as did the control cows. The percentage of the total iodine intake excreted through the milk was lower for the supplemented cows than for the control cows, suggesting that an exponential relationship exists between dietary iodine and the percentage appearing in milk. The concentration of iodine in the milk of the supplemented cows rose to a peak during week 22, after which it declined. This may have been a seasonal effect or may have been due to a decreasing metabolic level in the mammary gland as lactation declined.

The lack of response in milk yield, milk fat or total solids production is in contrast to the work of Hemken (27) and Lengemann (35) who reported increases in performances from iodine supplementation very similar to responses from thyroprotein feeding. The cows in these earlier studies had been on iodine deficient rations for 12 months previous to the start of supplementation. In addition, high levels of goitrogens were being fed. Under these conditions, it would be difficult to determine if increased milk yield and milk fat production were in response to iodine supplementation or merely a return to the normal state from one of deficiency. Milk production

TABLE VI

SECRETION OF IODINE IN MILK OF IODINE SUPPLEMENTED AND CONTROL COWS

Animal No.	Iodine Concentration				Iodine Secreted			
	1/28[a]	3/30	5/11	6/16	1/28	3/30	5/11	6/16
	(μg/kg)				(mg/day)			
I Supplemented								
305	172.2	160.5	336.4	235.3	4.476	3.790	6.490	4.006
311	207.1	160.5	347.3	354.9	5.319	3.415	7.589	8.314
321	185.6	326.5	493.6	297.2	5.854	9.320	12.741	7.799
370	213.9	351.0	533.8	333.6	6.502	10.360	14.062	8.345
433	180.2	394.5	563.0	333.6	4.445	8.272	10.246	5.831
444	227.3	397.3	570.3	325.1	6.338	10.519	8.316	5.092
Avg.	197.7	293.4	474.1	313.3	5.489	7.613	9.907	6.565
Controls								
410	10.8	13.6	76.8	19.3	.393	.381	2.029	.452
402	10.0	14.9	36.6	19.3	.267	.375	.722	.331
266	10.8	13.6	74.9	12.8	.298	.347	1.743	.271
315	26.9	10.9	54.8	24.6	1.192	.442	1.916	.788
339	21.5	20.4	95.1	35.8	.550	.508	2.190	.757
422	9.4	12.2	43.9	25.7	.374	.399	1.059	.579
Avg.	14.9	14.3	63.7	22.9	.512	.409	1.610	.530

[a]Sampling date.

is influenced by thyroid activity. It is possible a borderline deficiency could occur in cows consuming sufficient quantities of goitrogens in an otherwise low iodine diet. Over an extended period of time, this deficiency could decrease production.

The elevation of the iodine content in the milk of the supplemented cows is similar to the report of Alderman and Stranks (3) and Archibald (5). There is agreement that a close relationship exists between dietary iodine and milk iodine. The milk iodine levels of the control cows were under, or near, the deficiency level set by Alderman and Stranks (3) at 25 µg/l on three of the sampling dates. The fact, however, that no deficiency symptoms or supplemental responses were found would make the milk iodine level questionable as a measure of dietary iodine sufficiency. It would seem that the cow will not create a physiological deficiency of iodine by mammary excretion, in that when the level of blood iodine is low, very little iodine is excreted by the mammary gland.

Effect on Protein-Bound Iodine and Total Plasma Iodine

The plasma protein-bound iodine (PBI) levels measured at 6, 14, 22, and 26 weeks from the start of the experimental period are given in Table VII. The PBI level in the control cows remained relatively constant throughout the period. There was no statistically significant change among the four determinations made during the trial. The supplemented cows showed a significant ($P<.05$) increase in plasma PBI from the sixth to the fourteenth weeks. For the remaining determinations, no significant change was found in the plasma PBI of this group.

TABLE VII

PLASMA PROTEIN-BOUND IODINE OF IODINE SUPPLEMENTED AND CONTROL COWS

Iodine Supplemented					Control				
Cow	6[a]	14	22	26	Cow	6	14	22	26
(No.)	----------(µg/100 ml)----------				(No.)	----------(µg/100 ml)----------			
305	4.62	8.68	8.20	7.02	410	4.90	5.85	3.75	4.29
311	7.94	7.87	6.59	5.10	402	4.50	5.89	6.05	4.06
321	3.56	7.88	7.40	7.77	266	2.71	3.74	3.32	3.11
370	5.93	8.19	8.16	9.67	315	3.48	4.24	2.70	3.51
433	5.27	7.97	5.40	7.01	339	3.76	5.85	3.70	6.01
444	5.11	6.21	5.90	9.58	422	2.96	5.12	2.77	4.49
Avg.	5.41	7.80	6.94	7.69		3.72	3.51	3.72	4.26

[a]Week of trial.

The difference found between treatments was highly significant ($P<.01$). However, the value of the PBI as an indication of thyroid status under conditions of high iodine supplementation is questionable; since, as shown by Long (40), high blood inorganic iodide may cause the iodination of blood proteins which have no thyroid hormone properties or actions. Such iodinated proteins would, however, be measured in the standard PBI determinations. The PBI values reported from iodine supplementation have, in general, ranged higher than the values presented here. Hemken (27) reported values of 18.6 µg/100 ml four weeks after parturition; however, at that time, the cows had been receiving levels up to 240 mg/day for six to eight weeks before parturition. Long (40) reported values in the 60-80 µg/100 ml range. He also was feeding excessive amounts (up to 2.819 gm KI per day). There is sufficient evidence that the standard PBI determination can be influenced by increases in dietary iodine.

The total plasma iodine was determined in week 26 of the trial. The total plasma iodine data are given in Table VIII, along with the plasma protein-bound iodine. The difference in the total and the protein-bound iodine is labeled as non-protein iodine and probably consisted mainly of inorganic iodides. The percentage of the total iodine bound to protein, both thyroidal and other, in the supplemented cows was 33.4. In the control cows 76.8% of the total iodine was protein bound. The effects of this high level of circulating non-bound iodide on the control of thyroid secretion has not been fully elucidated, but the possibility exists that there could be a negative feedback on the hypothalamic release of thyroid stimulating hormone.

TABLE VIII

RELATIONSHIP OF TOTAL PLASMA IODINE TO PLASMA PROTEIN-BOUND IODINE OF IODINE SUPPLEMENTED AND CONTROL COWS

Iodine Supplemented				Control			
Cow	26 wk PBI	26 wk Blood I	Non-Protein Iodine	Cow	26 wk PBI	26 wk Blood I	Non-Protein Iodine
(No.)	----------(µg/100 ml)----------			(No.)	----------(µg/100 ml)----------		
305	7.02	35.96	28.94	410	4.29	5.53	1.24
311	5.10	10.52	5.42	402	4.06	5.27	1.21
321	7.77	22.36	14.59	266	3.11	4.45	1.34
370	9.67	18.51	8.84	315	3.57	3.81	0.24
433	7.01	31.00	23.99	339	5.01	7.24	1.24
444	9.58	19.89	10.31	422	4.49	7.01	2.52
Avg.	7.69	23.05	15.36		4.26	5.55	1.29

In addition, this plasma iodide saturation probably caused the elevated PBI artifact previously discussed.

Thyroid Secretion Rates

The estimations of thyroid secretion rate (TSR) are given in Table IX for each cow during each period of the trial. There was no significant difference in the rates between the paired cows in the first three determinations. The third period determination approached significance ($P<.10$). The fourth estimation, made during week 26 of the trial, revealed statistically significant differences between the groups ($P>.01$). The TSR of the supplemented cows rose between the first and second periods. Thereafter there was little change in the mean TSR for the supplemented cows. The control cows tended to decrease slightly in TSR from the second estimation through the end of the trial. However, no significance could be assigned to this decline. The value of 1.601 mg/day at 26 weeks agrees well with Mixner's (47) value of 1.619 mg/day at 6 months with supposedly normal cows. This would suggest several possibilities: (1) the control ration was not low enough in iodine to cause a deficiency, (2) the cows had not been on the treatment long enough to be deficient, or (3) with normal feeds the cow can conserve and recycle sufficient iodine to prevent a physiological deficiency in her own system. The relative constancy throughout the trial within each treatment agrees with Mixner's (47) postulation that TSR remains fairly constant throughout the lactation period.

As previously stated, the TSR was calculated as the product of

TABLE IX

ESTIMATED THYROID SECRETION RATES OF IODINE SUPPLEMENTED AND CONTROL COWS

Iodine Supplemented					Control				
	Week of Trial					Week of Trial			
Cow	6	14	22	26	Cow	6	14	22	26
(No.)	----------(mg/24 hr)----------				(No.)	----------(mg/24 hr)----------			
305	2.008	3.557	2.707	2.766	410	2.532	2.688	1.806	1.868
311	3.871	3.798	2.577	2.741	402	1.595	1.788	2.457	1.694
321	1.379	2.612	2.884	2.290	266	1.714	2.538	1.854	1.392
370	2.605	2.781	2.358	3.436	315	1.937	1.791	1.471	1.599
433	1.316	1.726	1.480	2.089	339	3.259	2.501	1.842	1.601
444	1.742	2.656	2.530	2.861	422	1.648	2.421	1.464	1.449
Avg.	2.154	2.855	2.423	2.697		2.114	2.289	1.816	1.601

the normal PBI (n), the volume of distribution of thyroxine (TVD), and the thyroxine fractional turnover rate per day (TFTR). The thyroxine fractional turnover rate per day was calculated by multiplying the hourly regression coefficient (b) of the logarithms of the PBI disappearance curve after thyroxine injection by 24 to give a daily rate. These regression coefficients established for each disappearance curve are given in Table X. From these data, it is evident that the percentage of total PBI degraded per hour was significantly lower ($P<.05$) for the supplemented cows during all estimation periods. One source of error herewith introduced may have been that the regression coefficient was calculated from the degradation of all PBI, regardless of source. Evidence has not been reported that thyroidal PBI and non-hormonal PBI are degraded in the same manner or that non-thyroidal PBI remains static. This fact, in conjunction with the fact that the normal or preliminary PBI determinations between groups may have varied in their respective proportions of T_4 activity, has caused doubt as to the accuracy of this method of TSR estimation in animals fed supplementary iodine or in which a high level of plasma inorganic iodide is present; as was the case in the supplemented cows. This fact, in conjunction with the possibility that the b values of the supplemented cows were not indicative of thyroxine degradation alone, resulted in the probably overestimation of TSR in the supplemented group.

Effect on Plasma Thyroxine Levels

An effort was made to determine the relationship of the gross differences in plasma protein-bound iodine to differences in circulating

TABLE X

REGRESSION COEFFICIENTS (b) OR THYROXINE TURNOVER RATE PER DAY OF IODINE SUPPLEMENTED AND CONTROL COWS

Iodine Supplemented					Control				
	Week of Trial					Week of Trial			
Cow	6	12	22	26	Cow	6	14	22	26
(No.)					(No.)				
305	.3018	.2525	.1783	.2124	410	.4382	.3253	.3313	.3049
311	.2818	.3169	.2623	.3548	402	.2275	.2142	.3037	.3013
321	.2895	.2175	.1969	.1012	266	.3571	.6095	.3961	.5732
370	.4643	.2175	.1920	.2725	315	.5600	.3446	.5178	.3120
433	.1865	.1412	.2343	.2160	339	.6670	.2876	.4133	.3840
444	.1534	.2847	.3217	.1518	422	.3994	.3294	.4159	.2099
Avg.	.2796	.2384	.2309	.2181	Avg.	.4415	.3518	.3963	.3476

thyroxine (T_4). Abbott Laboratories Tetrasorb-125 test for plasma thyroxine was carried out on the preliminary plasma samples taken at the 26-week TSR determination. These results are presented in Table XI. From these data, it was determined that there were, in fact, significant ($P<.05$) differences in circulating thyroxine (T_4) levels between the groups. There was a mean increase of 1.48 µg/100 ml of T_4 in the supplemented cows as compared to the control cows. These values (6.97 µg/100 ml for the supplemented and 5.49 µg/100 ml for the controls) are not in agreement with Lorscheider et al. (41) who reported 3.39 µg/100 ml plasma T_4 in cows producing at a comparable rate on a low iodine ration. They were close to the value of 5.64 µg/100 ml plasma T_4 reported by Lorscheider et al. (41) for low lactating cows. The difference in plasma T_4 at different production levels was the basis for Lorscheider et al. (41) to postulate the theory of a self-limiting iodine deficiency in lactating cows; however, no correlation was found in the present study between plasma T_4 level and milk yield level between cows. The lack of plasma T_4 determination earlier in lactation made the calculation of a milk yield-T_4 correlation on a within-cow basis impossible.

The plasma T_4 in the supplemented cows would convert to 4.55 µg/100 ml or 59.2% of the 7.69 µg/100 ml PBI. The plasma T_4 in the control cows would account for 3.59 µg of the 4.26 µg/100 ml PBI or 84.2%. This is further evidence that a significant portion of the plasma PBI in the supplemented cows was not T_4; however, the significant difference in circulating plasma T_4, as measured by the Tetrasorb-125,

TABLE XI

PLASMA THYROXINE (T_4) LEVELS IN SUPPLEMENTED AND CONTROL COWS AS DETERMINED BY TETRASORB-125 METHOD

Iodine Supplemented		Control		Iodine Supplemented Difference
Cow	Plasma T_4	Cow	Plasma T_4	Plasma T_4
(No.)	(µg/100 ml)	(No.)	(µg/100 ml)	(µg/100 ml)
305	7.45	410	5.45	2.00
311	8.40	402	5.80	2.60
321	5.85	266	5.85	0
370	6.40	315	6.40	0
433	6.40	339	5.25	1.15
444	7.30	422	4.20	3.10
Avg.	6.97		5.49	1.48

would indicate that increases in circulating thyroxine did occur with excessive iodine supplementation. The reason for this effect has not been elucidated.

CHAPTER V

SUMMARY AND CONCLUSIONS

Six pairs of high producing cows from the University of Tennessee Dairy herd were used to determine the effect on milk production and thyroid secretion rate of adding 100 mg of iodine daily to low iodine diets containing natural feedstuff goitrogens.

The 12 cows were started on the trial within one month after calving and continued for 26 weeks thereafter. No significant differences were observed in milk yield, fat, or total solids production. Milk iodine was increased significantly ($P<0.05$) in the cows receiving supplemental iodine. Approximately 34.0% of the dietary iodine appeared in milk of the control cows, compared to 9.3% for the supplemented cows. No adverse effects were observed in the cows on the low iodine diet which, according to most recognized standards, was iodine deficient.

Thyroid secretion rate estimations were made four times during the trial period. No significant differences were found between groups in the first three determinations. The fourth period estimates, however, were significantly different ($P<.01$). Also, plasma protein-bound iodine was significantly ($P<.01$) elevated at all periods in the iodine supplemented cows. This, however, was considered to be an artifact of high blood iodide and not representative of thyroidal PBI.

Plasma thyroxine was determined in the twenty-sixth week and found to be significantly higher ($P<.05$) in the supplemented cows.

This would indicate that there may have been increased thyroidal iodine binding with increased iodine intake. However, the failure of the low iodine cows to deplete their iodine stores through the mammary gland would suggest that the cow probably would not, under natural conditions, initiate a self-limiting iodine deficiency by mammary iodine excretion.

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VITA

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