SERUM THYROXINE RESPONSE TO THYROPROTEIN ADMINISTRATION IN THE DAIRY COW

Thesis for the Degree of M. S.

MICHIGAN STATE UNIVERSITY

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1972

ABSTRACT

SERUM THYROXINE RESPONSE TO THYROPROTEIN ADMINISTRATION IN THE DAIRY COW

By

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Twelve non-lactating dairy cows were fed 10 g thyroprotein daily for seven consecutive days. Blood serum samples were taken from six cows during the period of thyroprotein administration. The remaining six cows were sampled the week of withdrawal of thyroprotein. Serum was analyzed for protein-bound iodine and serum thyroxine. It was found that both protein-bound iodine and serum thyroxine increased with thyroprotein administration and decreased after withdrawal. Protein-bound iodine overestimated serum thyroxine in thyroprotein fed cows.

Of the twenty-one lactating diary cows used in longer term experiments, seven served as controls, seven received 15 g thyroprotein daily for 5 weeks, seven received 15 g thyroprotein daily for 13 weeks. Serum taken before, during and after treatment was analyzed for thyroxine levels.

Thyroprotein feeding caused the serum thyroxine level to increase from a control value of 6 μg/100 ml to a maximum value of over 13 μg/100 ml. Long term (13 weeks) and

short term (5 weeks) thyroprotein treatment maintained the thyroxine level above controls.

Withdrawal of thyroprotein caused a precipitate decline in serum thyroxine levels. The rate of decline, minimum level and subsequent return toward normal was not greatly affected by length of treatment.

Milk yields of thyroprotein-fed cattle were increased 13% over controls for six weeks, then declined to control values or below.

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By

Gary H. Shaw

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Physiology

1972

DEDICATION

To the memory of my very dear father

and to the greatest of Moms

ACKNOWLEDGMENTS

The author wishes to sincerely thank Professor E. P. Reineke for his kind guidance and assistance throughout all phases of this study. Without his confidence and encouragement this study would not have been possible.

Special thanks are due to Dr. E. M. Convey and Dr. H. A. Tucker for providing the blood serum samples and for helpful counseling, and to Dr. J. W. Thomas for the information on milk yields and for directing the management of the experimental cattle.

Appreciation is also due Dr. J. R. Hoffert for serving on my committee, critically reviewing and improving the manuscript.

Thanks are also due to Miss Marilyn Stoltzfus for assisting in the thyroxine analyses and to Miss Connie Worden for the typing of the rough drafts of this thesis.

Due to the academic and social interactions with the "guys upstairs" the time spent on this study was an enriching and pleasurable experience for which I am truly appreciative.

I am also indebted to the Michigan Agricultural Experiment Station for support on a research assistantship.

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INTRODUCTION

Thyroid hormones are required for normal milk production in lactating mammals (Kon and Cowie, 1961). Graham (1934a) first reported that secretions of the thyroid gland profoundly influenced lactational performance of dairy cows. Because of excessive costs and limited availability of thyroxine, utilization of this compound to enhance milk and milk fat production was not economically feasible. These restrictions were overcome when Reineke and Turner (1942) reported an *in vitro* method for production of thyroprotein which was an economic, orally active source of thyroid hormone.

For almost 30 years synthetic thyroprotein has been fed to dairy cattle, yet there have been no direct determinations of changes in serum thyroxine resulting from its feeding. This is due largely to the unavailability until recently, of a simple, accurate procedure for serum thyroxine determination.

The study to be reported was designed to determine changes in the serum thyroxine concentration of lactating dairy cattle before, during, and after inclusion of thyroprotein in the diet.

REVIEW OF LITERATURE

Altered thyroid states affecting milk production in dairy cows was first brought to attention by the work of Graham in 1934. He showed that feeding desiccated thyroid gland to thyroidectomized or normal cows could cause a rise in milk and milk fat production (Graham, 1934a). Graham also showed that injections of thyroxine caused increased milk and milk fat production (Graham, 1934b).

It was not until an inexpensive source of thyroactive substance was made available that widespread studies could be done. The development of iodinated casein (thyroprotein) (Reineke and Turner, 1942; Reineke _et al_., 1943) made available an inexpensive source of thyroactive substance. It was shown that thyroprotein contains thyroxine (Reineke and Turner, 1943a) specifically L-thyroxine (Reineke and Turner, 1943b) and many other iodine compounds (Mischler and Reineke, 1970).

Many studies were initiated concerning the effects of thyroxine and thyroprotein on dairy cattle, with special emphasis on milk production (Blater _et al_., 1949; Blaxter, 1952; Thomas, 1953; Moore, 1958).

The responses in cattle to thyroprotein are essentially the same as after the administration of thyroxine.

Oral administration of DL-thyroxine to dairy cows required approximately 16 times the amount given parentally to achieve the same lactational response (Bailey et al., 1949). Judging from its effect in causing weight losses in sheep, oral administration was about 12% as effective as parental administration of thyroxine (Turner and Reineke, 1946). From data obtained in sheep by the thyroxine substitution method (Wagner, 1959) it can be calculated that about 11% of the thyroxine in thyroprotein is absorbed when orally administered. The increased amount of thyroprotein necessary for oral administration is apparently not due to inactivation in the rumen (Thurner and Reineke, 1946) or to losses in the feces (Monroe and Turner, 1949).

The lactational response to thyroprotein varies greatly due to a number of factors. In the early studies differences in the potency and tests for potency of iodinated protein preparations could account for much of the variance (Reineke, 1946a). Even with standardized preparations there are many factors known to contribute to variations in milk productions. Age (Booth et al., 1947), breed (Blaxter, 1946), stage of lactation (Herman et al., 1938; Ralston et al., 1940), previous milk yields (Thomas et al., 1957), and nutrition (Thomas et al., 1949), affect the degree of response to thyroprotein.

It has been shown that thyroxine or thyroprotein increase the milk fat content (Graham, 1934; Reece, 1944), decrease the ascorbic acid (Chanda and Owen, 1952; Blaxter, 1952), and cause other changes in milk composition (Reece, 1944, 1950; Sen _et al_., 1954). Thyroprotein also causes a marked increase in the iodine content of milk (Bartlett _et al_., 1949). However, no significant amount of thyroxine has been found in milk (Reineke and Turner, 1944; Bruger and Selberbush, 1946).

As to be expected of a compound containing thyroxine, thyroprotein will cause a number of metabolic changes, including increased heart rate (Booth and Elvehjem, 1947; Swanson, 1951), decreased body weight (Swanson, 1951, 1954; Moore, 1958), and may induce heat stress (Moore, 1958). A decrease in general health may also be observed (Leech and Bailey, 1953).

There are several methods available for measuring thyroid gland activity and thyroid hormone levels.

In the substitution method, "thyroid secretion rate is determined as the minimum amount of thyroxine administered daily that will completely block the release of previously injected I^{131} taken up by the thyroid gland" (Post and Mixner, 1961a, p. 2265). This method, based on observations of Wolff (1951) and Perry (1951), first worked out for sheep (Henneman _et al_., 1952) was applied to dairy calves (Lewis et al., 1955; and Hendrich and Turner, 1964). In mature cattle it yields values ranging from 0.15-0.22 mg L-T_4/45.4 kg (Anderson, 1971).

Another method for measuring thyroid secretion rate (TSR) is the thyroxine pool turnover method. It was introduced by Ingbar and Freinkel (1955), applied to sheep (Freinkel and Lewis, 1957), and to cattle by Post and Mixner (1961a). In this method a small amount of radioactive thyroxine ($L\text{-}T_4I^{131}$) is injected, serum is then sampled at intervals and measurements are made of protein-bound iodine specific activity. In a corollary to this method (Mixner and Lennon, 1958) measurements of the decline in protein-bound iodine (PBI) are made following an initial injection of unlabeled thyroxine. From the decline in PBI or specific activity the thyroxine degradation rate can be calculated. In this method it is assumed that the degradation rate equals the secretion rate (Ingbar and Freinkel, 1955). Values of 1.42-1.53 mg T_4/day/cow (Anderson, 1971) for normal lactating dairy cows and 1.59-1.73 mg T_4/day/cow in mature nonlactating dairy cows (Post and Mixner, 1961a) were reported.

The substitution method and the pool turnover methods have been compared (Post and Mixner, 1961a; Bauman _et al_., 1969; Anderson, 1971). Anderson found that the substitution method overestimates the TSR by 12 to 22%.

Aside from measuring thyroid secretion rate, there are methods available to estimate serum thyroxine levels. One of the most commonly used is based upon the fact that most of the circulating thyroxine is bound to plasma protein

(Blincoe and Weeth, 1967). In this method (Barker and Humphrey, 1950), protein-bound iodine (PBI) is precipitated from serum and the iodine content of the precipitate is determined colorimetrically. This method has been applied to lactating dairy cattle to yield values of: 7.03 μg T_4/100 ml (Lewis and Ralston, 1953); 5.00 μg T_4/100 ml (Mixner et al., 1962); 5.66 μg T_4/100 ml (Bauman et al., 1969); and 7.47 μg T_4/100 ml (Anderson, 1971).

The most direct method of measuring serum thyroxine levels (used for the majority of the work in this study) uses the principle of competitive protein binding (Ekins, 1960). The method was altered by the use of a gel-filtration column (Murphy and Pattee, 1964) which was later replaced by an anion exchange resin (Murphy and Jachan, 1965) and finally modified by the use of a resin impregnated sponge (Nakajema et al., 1966; Kennedy and Abelson, 1967). The principle of this method is given under the section entitled materials and methods.

There are a number of other methods available to measure the level of thyroid function. A partial list would include: radioiodine uptake (Greer, 1951), [which has questionable value (Lodge et al., 1958)], thyroxine distribution volume and biological half-life (Bauman et al, 1969), and butanol-extractable iodine (Benotti and Benotti, 1963).

Some of the methods mentioned above have been applied to cattle receiving exogenous thyroxine. In two dairy bull calves, injections of 2.5 and 5.0 mg L-T_4 caused PBI levels to increase from 3.7 μg/100 ml to approximately 12 and 21 μg/100 ml respectively. Using the turnover method PBI was estimated to decline at a rate of 28% and 81%/hr in the same animals (Mixner and Lennon, 1958a). Plasma PBI levels were observed to increase to 10-14 mg/100 ml after the addition of L-thyroxine to the feed of 10 lactating cows. Removal of L-thyroxine from the feed caused PBI levels to drop below normal for a short period of time (Swanson and McFee, 1959).

Injections of thyroxine 50 and 100% above winter secretion rates caused the thyroxine half-life to decline from 2.32 days to 1.05 and 0.88 days respectively (Premachandra and Turner, 1961). Similarly injections of thyroxine 125 and 150% above normal thyroid secretion rates in dairy cattle caused the PBI levels to increase from 3.7 μg/100 ml to 7.1 and 9.1 μg/100 ml with a concurrent decrease in the half-life of thyroxine (Bauman <u>et</u> <u>al</u>, 1969).

Previous to the work to be presented herein, there is only one report of serum thyroxine levels after thyroprotein administration. Six cows fed 10 g thyroprotein for 6 days were found to have a mean serum thyroxine level of 9.66 μg/100 ml as measured by the competitive binding method (Etta and Reineke, 1971).

MATERIALS AND METHODS

Treatments

Preliminary Study

Twelve pregnant nonlactating Holstein cows and heifers were assigned to treatment groups. Six cows were fed 10 g thyroprotein[1] daily, beginning on day 1 and continued to receive thyroprotein through day 7. Thyroprotein was withdrawn on day 8. The other six cows were fed thyroprotein daily from day 8 through day 14. Daily blood samples were taken from all 12 cows and assayed for serum thyroxine (T_4) and protein-bound iodine (PBI) on days 8 through 14.

Final Study

Twenty-one lactating Holstein cows (see Appendix A) were assigned to one of three groups of seven cows each (A, B and C). Group A was fed 15 g thyroprotein (TP) on day 6 through day 97. Group B was fed 15 g thyroprotein on day 6 through 41. The controls (Group C) received no thyroprotein. All three groups were bled on day 0, day 3, and on subsequent dates as recorded in Appendix B.

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[1]Protamone supplied by Agri. Tech. Inc., Kansas City, Mo.

All cows were housed in stanchion barns of the Michigan State University Dairy Department. They were fed 10 lbs alfalfa haylage daily, corn silage to appetite (35-100 lbs/day/cow) and 1 lb grain per 2.2 lbs milk. The grain ration was adjusted every two weeks. The cows were milked twice daily. Milk weights were obtained for 5 days a week.

Thyroprotein was fed in dehydrated alfalfa carrier for the first 4 days, but because of incomplete consumption the method was changed such that 15 g of TP was mixed with 1 lb of the usual grain concentrate and fed in the morning, previous to any other feed. This resulted in complete TP consumption.

Blood Serum

Coccygeal vein blood was maintained at room temperature for 2-3 hrs then at 4°C for 9-10 hrs to allow clot formation. Thereafter, it was centrifuged at 2500 X g at 4°C for 10 min. in a Sorvall-RC3 centrifuge. The serum was aspirated and stored at -25°C until assayed.

Analytical Methods

Protein-bound iodine: Protein-bound iodine (PBI) was quantitated using the method of Barker and Humphrey (1950). Briefly, serum proteins are precipitated with zinc sulfate and NaOH, washed, dried, ashed, and the ash dissolved in an aqueous mixture of HCl and H_2SO_4. Iodide concentration of the resulting solution was estimated colorimetrically by its ability to catalyze the reaction of Ceric Ammonium Sulfate (yellow solution) + Arsenious acid to cerous sulfate (colorless solution).

Serum Thyroxine: Serum thyroxine (T_4) was measured by the Tetrasorb-125 method.[2] Briefly, serum proteins were precipitated with ethanol with the concurrent release of T_4 into the supernatant. A measured amount of the supernatant was then dried in polyproplene tubes. Thyroxine-binding globulin (TBG) labeled with $^{125}I\text{-}T_4$ was added, and allowed to equilibrate at room temperature for 10 min. An initial radioactivity count was then made. Endogenous T_4 displaces some of the ^{125}I-labeled T_4 from the TBG. A resin-impregnated sponge was then added to bind the labeled and unlabeled free T_4. The sponge was washed to remove the TBG-bound T_4. The ratio of the radioactivity counts in the washed sponge to the initial radioactivity count is proportionate to the endogenous T_4.

[2]Abbott Radio-Pharmaceuticals, North Chicago, Ill.

RESULTS

Preliminary Study

The preliminary study was initiated to determine to what degree and at what rate thyroprotein (TP) feeding would effect serum thyroxine levels. It was also used to test whether or not protein-bound iodine (PBI) determination could be used as an accurate measure of serum thyroxine levels in thyroprotein-fed cattle. Blood samples from twelve nonlactating dairy cows were taken on days 8 and 14 and subsequently analyzed for serum thyroxine using the PBI method and the competitive binding method. Data for the six cows fed 10 g of thyroprotein on days 8 through 14 inclusive are shown in Table I. Maximum individual T_4 values were obtained on days 9 or 10 (maximum mean value on day 10). The mean serum thyroxine levels then declined, but to a level that was still above the control level of day 8.

Six cows were fed 10 g of thyroprotein for seven days. Their T_4 values for the seven days following thyroprotein withdrawal are presented in Table II. The mean serum thyroxine levels declined from day 9, reaching a minimum on day 13, six days after withdrawal of thyroprotein.

TABLE I

Preliminary Thyroprotein Study: Initiation of Thyroprotein[a]

	Day 8	9	10	11	12	13	14
Mean[b] PBI T_4 µg/100 ml	7.25	15.12	10.61	10.88	10.53	10.59	11.31
S.E.	0.55	1.62	0.64	1.32	0.75	0.67	0.51
Mean[c] T_4 µg/100 ml	7.25	11.33	11.55	8.67	8.85	7.93	9.66
S.E.	0.66	1.01	0.88	0.81	0.53	0.43	0.38

[a]10 g thyroprotein/cow/day.

[b]Mean T_4 equivalent of the PBI for six cows run in duplicate.

[c]Mean T_4 for six cows determined by the Tetrasorb-125 method.

TABLE II

Preliminary Thyroprotein Study:
Withdrawal of Thyroprotein

	Day 8	9	10	11	12	13	14
Mean[a] PBI T_4 µg/100 ml	17.90	14.79	9.50	7.47	7.29[b]	6.41	6.34
S.E.	0.77	1.09	0.53	0.32	0.52	0.26	0.28
Mean[c] T_4 µg/100 ml	10.28	12.74	9.28	6.36	6.00	3.94	5.20
S.E.	0.83	1.09	0.86	0.37	0.43	0.44	0.43

[a]Mean T_4 equivalent of the PBI for six cows run in duplicate.

[b]Mean of five cows.

[c]Mean T_4 for six cows determined by the Tetrasorb-125 method.

A comparison of the serum thyroxine levels (Tables I and II) determined by the two different methods[1] shows that the protein-bound iodine method consistently overestimates the serum thyroxine level as compared with estimates derived from the competitive binding method. Thyroprotein contains about 7% total iodine, including iodide and a series of organic iodine compounds that are closely related to thyroxine (Mischler and Reineke, 1971). The protein-bound iodine method is considerably less specific (measuring all iodine compounds that will be precipitated with zinc hydroxide) than the competitive binding method (measuring exclusively thyroxine). Thus the protein-bound iodine method would be expected to yield higher values in thyroprotein-fed cows.

Final Study

Controls

Seven lactating dairy cattle receiving no thyroprotein were assigned to the control group. Blood samples were taken on the same days as the long and short term groups (days listed in Appendix B). Mean serum thyroxine levels are presented in Figures 1 and 2. Individual values are given under the appropriate heading in Appendix B.

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[1]Since thyroxine is 65.4% iodine by weight, PBI ÷ 0.654 = PBI thyroxine equivalent.

The mean serum thyroxine values can be fitted to a linear equation of the form $y = a + bx$, where y is the mean serum thyroxine value (in μg T_4/100 ml) for a particular day, b is the slope, x is the number of days, and a is the y-intercept in μg T_4/100 ml. The calculated y-intercept (a) was found to equal 5.43 μg thyroxine/100 ml, and the slope (b) was found to be 0.0108 μg thyroxine/100 ml/day ($P < 0.01$).

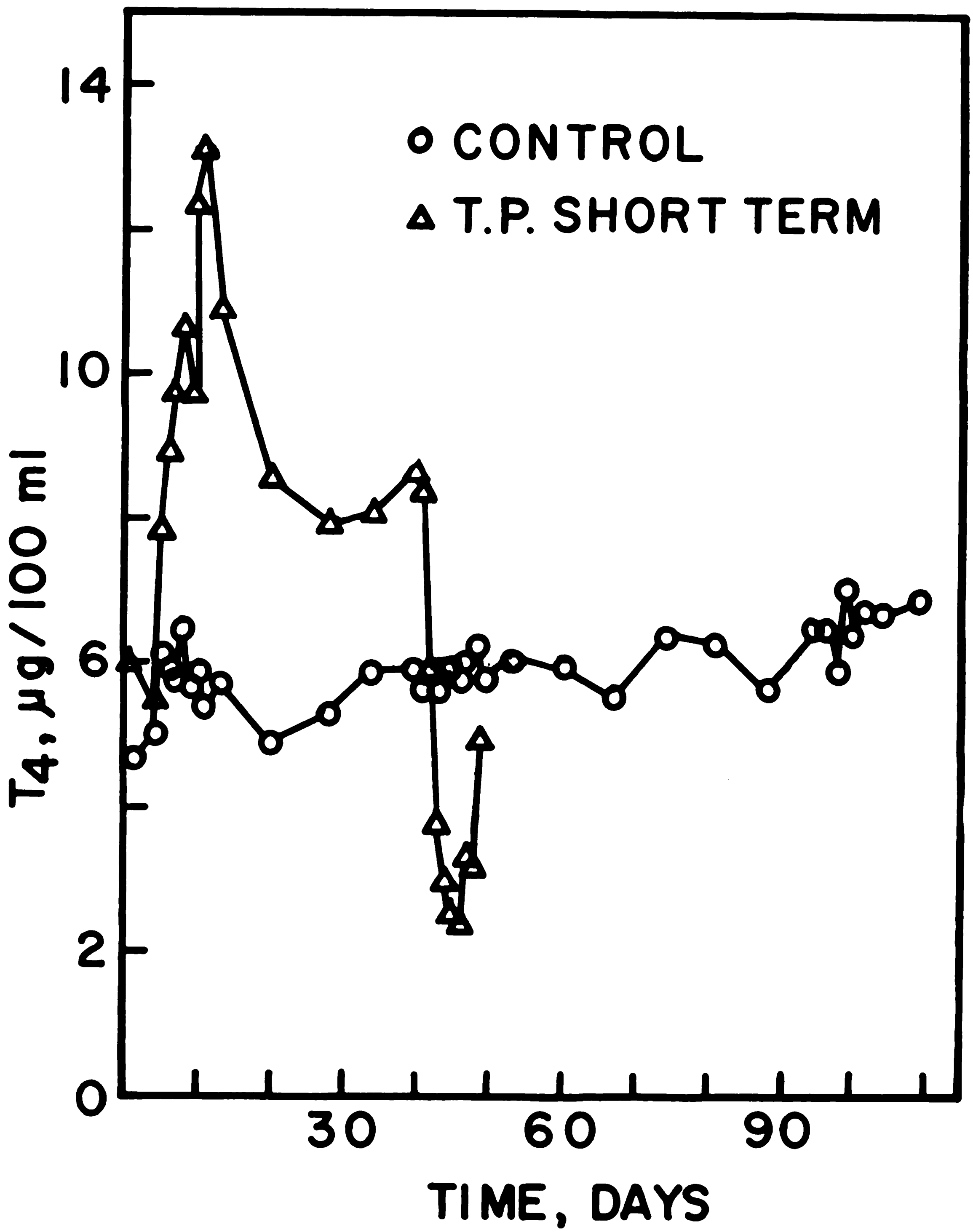
Short Term Thyroprotein Feeding

Seven different lactating dairy cattle were assigned to the short term feeding group (Group B). This group received 15 g thyroprotein on days 6 through 41 inclusive. They did not receive any thyroprotein on day 42 or on any subsequent day. Serum thyroxine (T_4) levels were determined on samples taken starting day 0 and on subsequent days as listed for this group in Appendix B. Mean serum thyroxine levels are presented in Figure 1. Individual values are given under the appropriate heading in Appendix B.

Pretreatment values determined for days 0 and 3, averaged 5.68 μg T_4/100 ml. On the morning of day 6 thyroprotein was fed; by sampling time that afternoon (approximately 12 hours later) the mean serum T_4 level had risen to 7.83 μg/100 ml. The serum T_4 level continued to rise, reaching a maximum of 13.13 μg/100 ml on day 12. On day 14 the concentration was down to about 11 μg T_4/100 ml; the T_4 level continued to decline, reaching a plateau of

Figure 1. Serum thyroxine levels in control and short term experimental cows.

Mean serum thyroxine concentration of control and short term thyroprotein (TP) fed lactating dairy cattle. Thyroprotein (15 g/day) fed days 6 through 41, inclusive. Each point represents mean of six animals.



14
10
6
2
0
T4, μg/100 ml
o CONTROL
Δ T.P. SHORT TERM
30
60
90
TIME, DAYS

Figure 1

approximately 8.3 μg T_4/100 ml. Thyroprotein was not fed on day 42; however, the serum T_4 level dropped only 0.32 μg/100 ml from the previous day. On day 43 the T_4 level was down to that of the controls; it continued to decline, going below controls to reach a minimum of 2.33 μg T_4/100 ml on day 47. The level then increased toward the control level, and by the last sampling day (day 52) had reached 4.93 μg T_4/100 ml.

Long Term Thyroprotein Feeding

Another seven lactating dairy cattle were assigned to the long term feeding group. This group received 15 g thyroprotein on days 6 through 97. They did not receive any thyroprotein on day 98 or on any subsequent day. Mean serum thyroxine levels are presented in Figure 2 and individual values are given in Appendix B.

Pretreatment values determined for days 0 and 3, averaged 5.58 μg T_4/100 ml. On the morning of day 6 thyroprotein was fed. By sampling time that afternoon (approximately 12 hours later) the serum T_4 level had risen to 7.28 μg/100 ml. The serum T_4 level continued to rise, reaching a maximum of 13.98 on day 12. On day 14 the level was down to 12.49 μg T_4/100 ml; it continued to decline reaching a local minimum of 7.76 on day 29. During the remaining time, while thyroprotein feeding was continued, the cows' mean serum T_4 level fluctuated markedly, but it

Figure 2. Serum thyroxine levels in control and long term experimental cows.

Mean serum thyroxine concentration of control and long term thyroprotein (TP) fed lactating dairy cattle. Thyroprotein (15 g/day) fed days 6 through 97, inclusive. Each point represents mean of six animals.

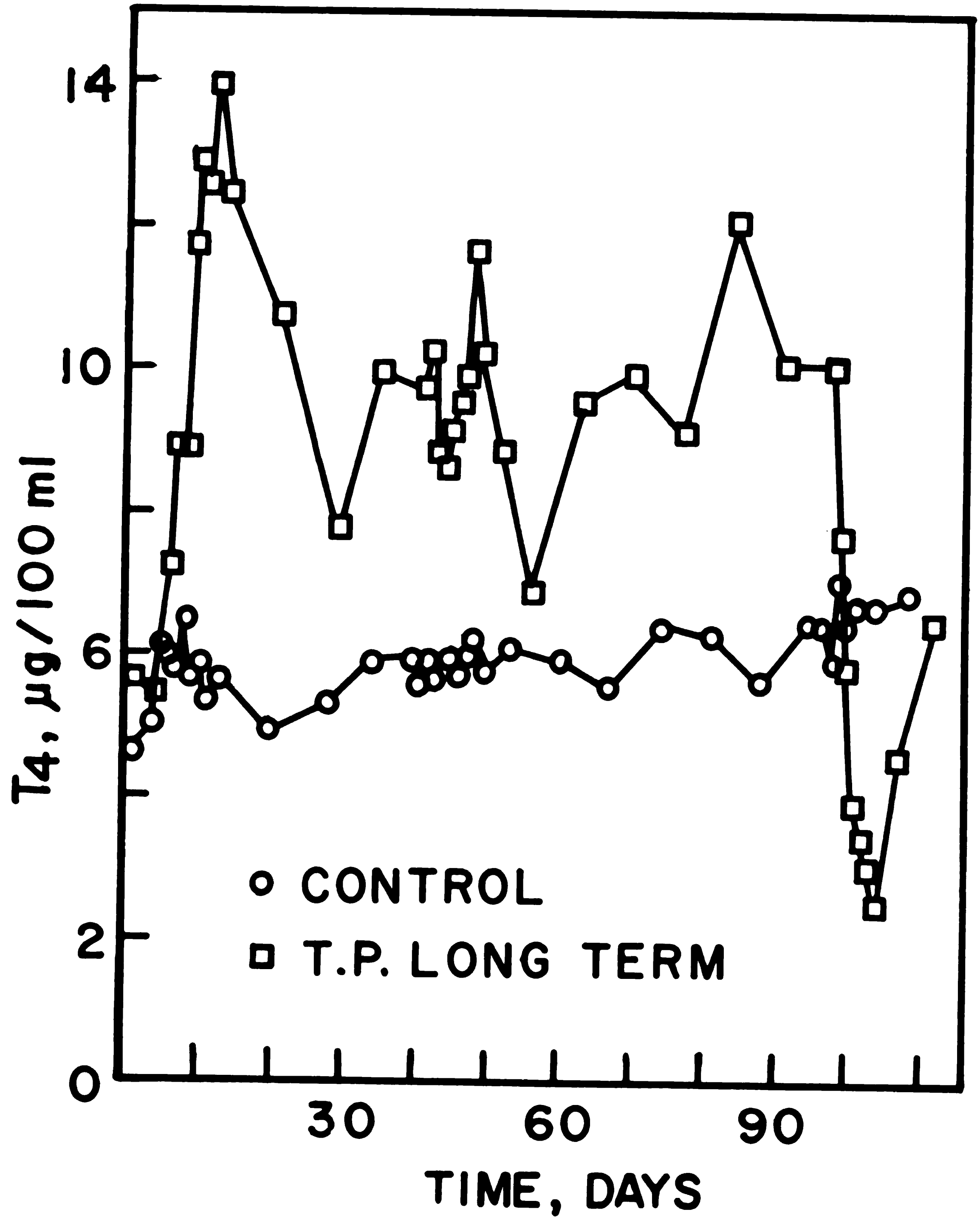


Figure 2

never dropped to control level nor did it ever reach the height of the first maximum level.

On day 98 no thyroprotein was fed; the mean serum level was 10.09 μg T_4/100 ml. The level had dropped to 7.65 μg/100 ml on day 99 and continued to decrease, going below controls to reach a minimum of 2.51 on day 104. The level then increased to a value not significantly different from the control level of day 112.

Milk Production

Most studies of thyroprotein administration have been concerned with its effects on milk production. For this reason milk yields from the cows in groups A, B, and C have been included in Appendix C.

Changes in milk production with time and thyroprotein administration for groups A, B, and C are presented in Table 3 and Figure 3. The values show the percent milk yield for a given time period as compared with a control period (9/20/71 to 10/3/71).

For the first two weeks of treatment, group A responded to thyroprotein feeding by increasing their milk production to 102.2% of their control level. They maintained a higher percent milk yield (as compared with Group C) until the two week period beginning 12/7/71. From 12/7/71 until 1/3/72, although continuing to receive thyroprotein, the percent milk yield then dropped below that of the

TABLE III

Milk as Percent of Pretreatment Level

Date	Group A	Group B	Group C
	(%)	(%)	(%)
9/20/71-10/3/71	100.0	100.0	100.0
10/4/71	102.2[a]	104.7[a]	93.6
10/18/71	95.5[a]	100.9[a]	84.2
11/1/71	97.0[a]	99.8[a]	89.3
11/9/71	90.1[a]	63.6	82.9
11/23/71	75.3[a]	76.9	72.6
12/7/71	70.1[a]	76.3	76.5
12/21/71	63.2[a]	68.7	69.5
1/4/72	48.6	65.0	66.9

[a]15 g thyroprotein fed during this time period.

Figure 3. Milk and percent fat response to thyroprotein.

Responses of milk production and fat content to administration of thyroprotein (TP) in lactating dairy cattle. Thyroprotein dose: 15 g/day. Each point represents mean response of six animals.

———————— control

---O---O--- long term (Group A) TP fed days 6-97

---X---X--- short term (Group B) TP fed days 6-41 control group. For the two week period beginning on 1/4/72, during which thyroprotein had been withdrawn, the percent milk yield was well below that of the control group.

During the first two weeks of treatment group B responded to thyroprotein feeding by increasing their milk production to 105% of their control level. They maintained their milk production at approximately 100% of their control levels for the next three weeks of thyroprotein feeding. For the first two-week period that group B did not receive thyroprotein, their milk production fell well below that of the control group. After this initial decline, their milk production increased so as to parallel that of the control group for the remainder of the study.

The milk production of the control group decreased with time, as is to be expected of cattle in the declining stage of lactation.

There was a sustained elevation in milk fat (see Figure 3).

DISCUSSION

Milk Production

During the two week period preceding thyroprotein feeding, milk production for groups A and B was quite close (102% and 99%) to that of group C (see Appendix C).

The milk production during the first two weeks of treatment, for groups A and B were 111% of group C. This compares favorably with the 109.9% of control reported for cows receiving thyroprotein at peak lactation (Tucker and Reece, 1961). Premachandra and Turner reported that the maximum response occurred in 3-5 days (1959). In a similar study they also reported a 27% increase (1962a); however, in these studies thyroxine was injected at 50% above the normal thyroid secretion rate.

During the second two week period of treatment the milk yields of groups A and B were 116% and 118% of group C, but by the third two week period their milk production was down to 111% of group C.

Although group A continued to receive thyroprotein for the remainder of the 13 weeks, its milk production dropped toward, then below that of the control group. This decline toward control has been reported to occur 16-18 weeks after initiation of thyroprotein feeding (Schmidt et al., 1971).

Group B received no thyroprotin after November 9, 1971. During the first two weeks after thyroprotein withdrawal their milk production dropped to 76% of the controls. This was a 37% decrease compared to the previous two week period. In a similar experiment Reece (1947) reported a 34% decrease after two weeks of withdrawal. This decline in milk production was reversed during the next sampling period. This rebound effect has been reported to occur after 10-12 days (Premachandra and Turner, 1962b). When thyroprotein was withdrawn from group A, their milk production declined in a manner similar to group B. However, they were dried off before any rebound effect could be noted.

The conclusion is that thyroprotein administration is only good for short term stimulation of milk production, as suggested by Thomas et al. (1954).

Percent milk fat was shown to increase in groups A and B during thyroprotein feeding. The elevation was maintained in group B even after withdrawal of thyroprotein.

Preliminary Study

The preliminary study showed that administration of thyroprotein to cattle would cause marked elevation in serum thyroxine levels. It also demonstrated that protein-bound iodine determination could not be used as an accurate measure of serum thyroxine levels. The competitive binding method was used exclusively in the final study, for this reason. The inability of the protein-bound iodine method to give reliable T_4 values is due probably to the many iodinated compounds present in thyroprotein (Mischler and Reineke, 1970). The inaccuracy is not due to large iodine losses occurring during the chemical processing of the serum (Post and Mixner, 1961b) nor does prolonged serum storage time contribute much variance (Lennon and Mixner, 1957).

Final Study

Control

The mean serum thyroxine levels for the control cows increased with time. This was found to be linear and may be expressed by the equation:

$$\text{mean serum } T_4\text{, } \mu g/100 \text{ ml} = (0.0108 \ \mu g \ T_4/100 \text{ ml/day}) \text{ (number of days)} + 5.43 \ \mu g \ T_4/100 \text{ ml}$$

Using this equation the calculated mean serum thyroxine levels on day 100 would be equal to:

$$(0.0108 \ \mu g \ T_4/100 \text{ ml/day}) \ (100 \text{ days}) + 5.43 \ \mu g \ T_4/100 \text{ ml}$$
$$= 1.08 \ \mu g \ T_4/100 \text{ ml} + 5.43 \ \mu g \ T_4/100 \text{ ml}$$
$$= 6.51 \ \mu g \ T_4/100 \text{ ml.}$$

This compares well with the experimental value found for day 100; 6.41 μg/100 ml.

Lorscheider (1970) showed that in the rat increased lactation was accompanied by a decrease in serum thyroxine levels. He also showed that cattle at peak lactation had thyroxine levels 59% of dry cattle but by the end of lactation the serum thyroxine had risen to that of the nonlactating cattle.

The range of mean serum thyroxine values for the control group was 4.68 to 7.10 μg/100 ml. This is in agreement with values in the literature. Bauman and coworkers (1969), sampling six cows, reported a mean protein-bound iodine value of 3.7 μg/100 ml, which is equivalent[1] to a serum thyroxine level of 5.66 μg/100 ml. Other values reported are (after conversion to serum thyroxine): 5.00 μg/100 ml (Mixner et al., 1962) and 7.03 μg/100 ml (Lewis and Ralston, 1953).

Treated

The long term thyroprotein-fed cows (group A) and the short term thyroprotein-fed cows (group B) received the same treatment initially. As expected the two groups initially responded in the same manner. Groups A and B reached their maximum levels of 13.98 μg/100 ml and 13.13 μg/100 ml

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Since thyroxine is 65.4% iodine by weight PBI μg/100 ml ÷ 0.654 = thyroxine equivalent μg/100 ml.

on day 12. The maximum levels for groups A and B were not significantly different from each other but were highly significantly greater than control levels ($P < 0.01$).

Groups A and B declined from their maximum to reach local minimums of 7.76 and 7.92 μg T_4/100 ml on day 29. This decline from maximum could be due to the saturation of the thyroxine-binding globulin, increased activity of metabolizing enzymes, and de nova synthesis of additional metabolizing enzymes. Another factor to be considered here is that the unusually high levels of T_4 in the serum would inhibit the release of thyroid stimulating hormone from the pituitary, which in turn would cause inhibition of release of T_4 from the thyroid. There is much evidence for this mechanism. In rats administration of thyroprotein was shown to cause suppression of the thyroid as measured by radioiodine output, radioiodine uptake, radioiodine biological half-time, thyroid weight, and thyroid histology (Stewart, 1966). In cattle the suppression of the thyroid via administration of T_4 is the basis of the substitution method described in the literature review (see Premachandra and Turner, 1961).

The rates of increase in serum thyroxine from day 6 to day 12 for groups A and B were 1.12 and 0.88 μg/100 ml/day. Since at this point both groups had received the same treatment, their rates of increase may be combined yielding an average increase of 1.00 μg T_4/100 ml/day. Thyroprotein contains approximately 1% thyroxine (Mischler and Reineke, 1971) of which approximately 10% is claimed to be absorbed when orally administered (Mixner and Lennon, 1958b), therefore feeding 15 g thyroprotein should be equal to parenteral administration of 15 mg T_4. If the volume of distribution for T_4 is estimated to be 60 liters (Mixner et al., 1962), 15 g thyroprotein/day should cause an increase in serum T_4 of 25 μg/100 ml/day. This is considerably different from the 1 μg/100 ml/day found experimentally.

There are two factors that could be responsible for this gross difference. First, in the study that determined the oral absorption value of 10%, serum T_4 levels were estimated using the protein-bound iodine method. Earlier in this paper it was shown that protein-bound iodine determinations are not an accurate measure of serum thyroxine levels. This error is compounded by the fact that in the study under question (Mixner and Lennon, 1958a) large amounts (25 g) of thyroprotein were fed.

Secondly, thyroxine, at levels above normal, tends to be cleared faster than at low or nomal levels. Evidence for this can be seen in that there was a progressive decrease in the rate of increase of serum thyroxine levels as they approached the maximum. This may be due to an increase in the activity of the metabolizing enzymes. The increased clearance at high levels would be enhanced by the fact that the thyroxine-binding globulin is soon saturated

(Etta and Reineke, 1971) and cannot bind the additional T_4 present.

During the time from day 30 until thyroprotein was withdrawn groups A and B maintained a relatively constant serum thyroxine level. Since they were absorbing approximately 15 mg T_4/day, in order to maintain a plateau, they must have been clearing 15 mg T_4/day. This reaffirms the statement that at high levels serum thyroxine is cleared faster than at normal levels. A decrease in the half-life ($T_{1/2}$) of thyroxine should result from an increase in the clearance rate. Premachandra and Turner (1961) showed that in normal cattle the $T_{1/2}$ of thyroxine was 2.32 days. However, if thyroxine was injected at 150% of the normal thyroxine secretion rate the $T_{1/2}$ decreased to 1.05 days; at 200% of thyroxine secretion rate the $T_{1/2}$ was reduced further, to 0.88 days. Similar results have been reported by Bauman _et al_. (1969).

From day 30 until day 97 group A received a constant amount of thyroprotein daily. Instead of showing a level plateau their serum thyroxine levels fluctuated markedly. An inspection of the individual records shows that the fluctuations were asynchronous. An autocorrelation test was performed on the plateau region to test for cyclicity. The only significant results found were that the serum thyroxine levels on a given day were significantly correlated to the levels of the previous day.

The wide fluctuation observed might be due to variations in the length of time required for the thyroxine to reach absorptive surfaces in the gastrointestinal tract. Thyroprotein has been shown to have an effect on the gastrointestinal tract of the cow, causing a decrease in the gastrointestinal fill (Swanson, 1954).

When thyroprotein was withdrawn from groups A and B, the serum thyroxine level declined in similar manners. Both groups showed little, if any, decline in thyroxine on the day of withdrawal. However, by the second full day off thyroprotein the level had dropped approximately 2.5 μg/100 ml. Both groups then declined rapidly, reaching minimum levels below control of 2.51 and 2.35. Group B reached its minimum 6 days after the last thyroprotein feeding. Group A required 7 days to reach its minimum, but group A was at a higher serum thyroxine level when thyroprotein was withdrawn. The rates of decline for groups A and B from the first day off thyroprotein to the minimum of 1.08 μg T_4/100 ml/day and 1.00 μg T_4/100 ml/day, were not significantly different from each other.

After reaching the minimum level both groups increased their serum thyroxine levels toward normal. This showed that even after prolonged suppression of the thyroid by exogenous thyroxine the thyroid-pituitary system was still able to resume function, and did so in not longer than 2 weeks. Bauman et al. (1965) showed that the thyroid of cattle previously suppressed by thyroxine injection would resume the uptake of labeled iodine when the thyroxine injections were discontinued.

One interesting theoretical discussion of effects of thyroprotein administration on the serum thyroxine levels in cattle has been given by Pipes et al. (1959). In this work it is claimed that in a 1000 lb cow secreting 2 mg T_4/day the addition of 15.0 g thyroprotein/day would cause drastic changes in the serum thyroxine levels. Specifically, they claimed that under these conditions the total pool of thyroxine would increase from 5.1 mg to 38.6 mg. If it is assumed that the volume of distribution remains constant, then the concentration of thyroxine in the serum would increase approximately sevenfold. Thus, if the original serum thyroxine level was 6.0 μg/100 ml the level after equilibration would be 45.4 μg/100 ml.

In this study 15 g thyroprotein was shown to cause the mean serum thyroxine levels to increase to approximately 14 μg/100 ml. Thus the theoretical estimation was almost 3 times the actual level achieved. The theoretical model required 23 days to reach equilibration or 13 days to reach 99% of the equilibration value. They also stated that the equilibration level would be maintained at the original peak level. In this study the level at which the plateau was maintained was reached in two days. However, it was shown that the level continued to rise to a maximum, then declined to the plateau. Thus equilibration was not obtained until approximately 3 weeks after the initiation of thyroprotein feeding, even though the level was reached in two days. The theoretical model did predict correctly the rapid decline in thyroxine, and the drop below control levels which was to be expected considering the well known negative feedback mechanism. It appears then, that a theoretical model may be of some use when there is incomplete data, but that it may also be very misleading.

SUMMARY

It was found that protein-bound iodine determinations are not good estimates of serum thyroxine levels in thyroprotein-fed cattle.

Lactating dairy cattle fed 15 g thyroprotein showed a transient increase in milk production, followed by a decrease after withdrawal.

Thyroprotein feeding caused sustained elevated serum thyroxine levels in dairy cattle. The decline in serum thyroxine levels after withdrawal was found to be essentially the same in cattle fed thyroprotein for 5 weeks or 13 weeks.

Resumption of normal thyroid function after up to 13 weeks of thyroprotein feeding occurred within approximately two weeks.

Normal post-peak lactating dairy cattle had increasing serum thyroxine levels as lactation progressed.

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APPENDIX A

REPRODUCTION HISTORY OF THE COWS

IN THE FINAL STUDY

APPENDIX A

REPRODUCTION HISTORY OF THE COWS IN THE FINAL STUDY

Cow Number	Group	Date Fresh	Milk[a] per lb.	Lactation Number	Pregnancy State
832	A	4/27/71	41.5	4	Due 4/24/72
833	C	4/30/71	63.5	4	Bred 8/27/71
873	C	4/30/71	43.5	5	Bred 9/13/71
906	B	3/17/71	44.0	3	Bred 9/20/71
935	A	5/5/71	64.0	3	Open
939	A	6/16/71	55.5	3	Bred 9/9/71
958	B	6/24/71	43.0	4	Bred 9/26/71
1008	A	4/18/71	46.0	2	Open
1010	A	4/16/71	42.5	2	Bred 9/11/71
1034	A	7/5/71	50.5	1	Open
1037	B	6/15/71	60.5	2	Bred 9/4/71
1061	B	4/30/71	49.0	1	Bred 8/30/71
1072	B	5/15/71	42.0	1	Due 4/14/72
1073	C	3/19/71	42.0	1	Due 4/28/72
1074	B	3/12/71	38.5	1	Due 3/4/72
1075	C	6/15/71	50.5	1	Bred 9/26/71
1078	C	3/25/71	39.0	1	Due 3/27/72
1082	C	7/6/71	48.0	1	Open
1087	B	6/27/71	41.5	1	Bred 8/31/71
1091	C	6/8/71	49.0	1	Bred 9/10/71
1093	A	6/4/71	42.0	1	Bred 9/15/71

[a]Determined on 9/14/71.

APPENDIX B

SERUM THYROXINE LEVELS

APPENDIX B

SERUM THYROXINE LEVELS (μg/100 ml)
Long Term Feeding (Group A)

	Cow #									
Date	832	935	939	1008	1010	1034	1093	Day	Mean	S.E.
9/28/71	6.30	5.51	5.21	6.60	5.04	6.18	4.87	0	5.67	0.258
10/1/71	4.26	3.81	4.29	6.86	8.25	5.63	5.31	3	5.48	0.604
10/4/71	7.85	4.55	9.17	7.70	8.06	6.83	6.88	6	7.28	0.552
10/5/71	7.80	6.47	9.92	9.73	9.32	10.30	8.82	7	8.91	0.510
10/6/71	8.55	7.79	9.19	7.29	10.16	10.30	9.07	8	8.91	0.426
10/7/71	12.00	10.58	12.60	11.99	13.21	9.31	12.77	9	11.78	0.519
10/8/71	11.72	13.00	11.09	11.95	11.76	14.74	16.10	10	12.91	0.699
10/9/71	10.35	11.92	10.35	13.45	12.05	13.17	16.72	11	12.56	0.828
10/10/71	15.33	14.13	9.90	13.66	13.96	14.06	16.81	12	13.98	0.796
10/12/71	13.18	13.11	9.54	9.59	14.73	12.17	15.14	14	12.49	0.847
10/19/71	10.51	13.11	7.52	9.11	10.77	9.63	14.73	21	10.77	0.924
10/27/71	7.66	5.11	4.40	6.80	11.75	6.51	12.06	29	7.76	1.147
11/2/71	12.05	11.22	8.38	10.76	8.76	5.78	12.77	35	9.96	0.924
11/8/71	10.53	9.34	8.06	8.13	9.72	10.44	11.71	41	9.70	0.502
11/9/71	11.80	8.08	7.67	8.74	11.85	11.90	12.54	42	10.24	0.874
11/10/71	11.12	8.53	5.68	7.39	8.60	12.66	8.46	43	8.89	0.893
11/11/71	7.73	8.67	5.43	8.39	10.41	9.55	10.18	44	8.62	0.646
11/12/71	7.76	9.88	4.75	8.25	10.79	9.68	13.11	45	9.17	0.992

Long Term Feeding (Group A)--Continued

Date	Cow # 832	935	939	1008	1010	1034	1093	Day	Mean	S.E.
11/13/71	8.46	9.89	6.65	10.06	9.87	10.18	11.57	46	9.53	0.589
11/14/71	8.42	9.69	7.51	11.36	9.16	12.14	11.03	47	9.90	0.635
11/15/71	12.57	12.49	7.49	11.12	11.29	11.04	15.61	48	11.66	0.917
11/16/71	9.94	8.74	6.74	12.95	8.38	10.63	14.03	49	10.20	0.975
11/19/71	8.53	9.26	7.07	8.07	10.47	8.79	9.68	52	8.87	0.411
11/23/71	6.94	5.50	4.60	6.13	6.31	10.02	8.51	56	6.86	0.699
11/30/71	8.33	9.64	5.64	6.96	11.61	11.38	13.13	63	9.53	1.022
12/7/71	10.71	9.52	7.36	9.95	10.49	8.20	13.17	70	9.91	0.710
12/14/71	8.07	8.00	8.13	9.39	6.77	12.03	11.44	77	9.12	0.737
12/21/71	13.50	11.07	9.24	13.79	12.42	13.27	11.38	84	12.10	0.618
12/28/71	13.22	8.22	10.48	5.02	7.59	11.99	13.86	91	10.05	1.227
1/4/72	15.61	11.35	8.02	3.12	6.90	13.49	12.17	98	10.09	1.626
1/5/72	11.54	6.92	6.17	8.12	5.19	8.44	7.15	99	7.65	0.772
1/6/72	10.69	4.81	4.16	5.24	3.93	7.87	4.03	100	5.82	0.961
1/7/72	6.68	3.33	1.64	5.86	1.87	4.18	3.78	101	3.91	0.711
1/8/72	5.41	2.55	1.69	6.92	2.04	3.24	2.14	102	3.41	0.737
1/9/72	3.96	1.19	1.47	8.12	1.33	2.86	2.04	103	3.00	0.932
1/10/72	2.34	1.72	2.75	6.69	1.29	1.32	1.47	104	2.51	0.726
1/13/72	5.08	3.29	7.64	9.35	2.64	0.96	2.84	107	4.55	1.132
1/18/72	3.12	6.61	6.91	8.49	7.83	5.67	6.63	112	6.47	0.655

SERUM THYROXINE LEVELS (μg/100 ml)
Short Term Feeding (Group B)

	Cow #									
Date	906	958	1037	1061	1072	1074	1087	Day	Mean	S.E.
9/28/71	6.59	4.70	6.69	7.10	6.22	5.34	5.10	0	5.96	0.345
10/1/71	4.69	4.27	6.95	6.74	6.30	5.13	3.72	3	5.40	0.480
10/4/71	8.17	6.79	9.58	9.13	7.17		6.15	6	7.83	0.554
10/5/71	8.93	9.30	12.14	9.81	8.36	8.28	6.50	7	9.05	0.651
10/6/71	8.33	10.31	11.65	10.35	9.31	8.17	9.88	8	9.71	0.463
10/7/71	7.28	9.63	11.37	13.35	12.87	8.69	11.13	9	10.62	0.834
10/8/71	7.82	9.30	10.26	12.23	11.84	8.15	8.31	10	9.70	0.678
10/9/71	12.84	9.98	10.60	14.46	12.30	12.30	14.07	11	12.36	0.624
10/10/71	13.12	11.56	12.12	15.01	13.16	13.20	13.77	12	13.13	0.421
10/12/71	11.89	7.16	13.55	12.91	5.47	11.99	13.07	14	10.86	1.209
10/19/71	10.34	6.24	8.75	10.38	3.00	10.04	10.82	21	8.51	1.092
					Excluding Cow 1072				(9.43)	(0.699)
10/27/71	9.68	9.73	6.59	6.82	10.85	4.17	7.59	29	7.92	0.874
11/2/71	8.84	7.95	6.96	8.07	9.32	4.70	10.61	35	8.06	0.711
11/8/71	9.27	7.98	9.43	6.96	10.14	10.60	6.23	41	8.66	0.621
11/9/71	9.80	6.75	6.18	7.27	11.60	12.20	4.61	42	8.34	1.090
11/10/71	7.55	5.20	4.16	4.72	7.65	8.11	3.39	43	5.83	0.721
11/11/71	4.45	3.63	3.24	1.81	5.96	4.62	2.31	44	3.72	0.540
11/12/71	3.66	2.34	3.52	2.64	3.31	3.14	2.16	45	2.97	0.223
11/13/71	3.17	2.73	3.67	1.91	2.22	2.30	1.60	46	2.51	0.274
11/14/71	2.13	2.92	5.06	1.37	2.04	1.65	1.29	47	2.35	0.497
11/15/71	2.75	4.51	6.45	1.43	1.82	2.74		48	3.28	0.768
11/16/71	3.25	5.60	7.18	1.38	1.37	1.48	2.05	49	3.19	0.880
11/19/71	5.19	6.84	6.83	2.57	2.88	4.62	5.58	52	4.93	0.648

SERUM THYROXINE LEVELS (μg/100 ml)
Controls (Group C)

Date	Cow # 833	873	1073	1075	1078	1082	1091	Day	Mean	S.E.
9/28/71	4.64	4.73	5.22	4.00	5.03	4.43	4.71	0	4.68	0.150
10/1/71	3.93	5.75	4.49	4.56	7.09	4.62	4.73	3	5.02	0.401
10/4/71	5.60	6.17	5.86	5.46	7.50	5.47	6.90	6	6.14	0.297
10/5/71	4.85	6.84	5.42	5.10	7.95	4.05	7.39	7	5.94	0.549
10/6/71	5.26	4.79	5.90	5.34	7.50	6.08	5.56	8	5.78	0.329
10/7/71	4.99	6.23	7.04	6.62	9.38	4.93	6.03	9	6.46	0.570
10/8/71	4.26	5.26	6.78	5.34	7.30	6.98	3.43	10	5.62	0.554
10/9/71	4.32	6.43	5.93	5.54	7.34	6.35	5.17	11	5.87	0.369
10/10/71	5.30	6.56	5.86	4.92	6.68	3.39	4.88	12	5.37	0.429
10/12/71		6.97	6.22	4.63	6.97	4.11	5.38	14	5.71	0.492
10/19/71	4.27	6.61	5.13	5.23	4.84	3.95	4.49	21	4.93	0.329
10/27/71	4.93	5.98	6.10	5.16	6.28	4.92	3.93	29	5.33	0.318
11/2/71	4.11	6.77	6.84	6.24	7.48	5.61	4.39	35	5.92	0.483
11/8/71	3.06	6.49	6.71	5.26	8.95	6.26	5.25	41	5.99	0.677
11/9/71	4.71	5.84	6.28	5.00	7.04	4.44	5.73	42	5.58	0.349
11/10/71	5.53	5.43	5.79	5.00	6.57	6.67	6.39	43	5.91	0.242
11/11/71	5.87	6.49	4.22	4.80	7.49	4.76	5.57	44	5.60	0.428
11/12/71	4.49	6.43	7.54	5.44	7.43	4.93	4.87	45	5.88	0.476
11/13/71	4.84	6.03	6.92	5.39	8.09	5.58	4.98	46	5.98	0.440
11/14/71	5.62	6.36	5.47	5.40	7.04	4.99	5.03	47	5.70	0.282

Control Group (Group C)--<u>Continued</u>

Date	Cow # 833	873	1073	1075	1078	1082	1091	Day	Mean	S.E.
11/15/71	6.05	6.29	7.08	4.56	7.46	5.10	5.23	48	5.97	0.404
11/16/71	5.23	6.69	6.74	6.98	7.65	4.56	6.20	49	6.29	0.403
11/19/71	3.54	6.96	6.26	5.24	8.42	5.71	4.24	52	5.77	0.623
11/23/71	4.26	7.65	4.90	4.99	7.13	9.06	4.76	56	6.11	0.691
11/30/71	4.34	6.20	7.04	5.43	8.85	4.41	5.66	63	5.99	0.597
12/7/71	2.89	7.40	6.14	5.91	7.33	2.53	6.95	70	5.59	0.775
12/14/71	5.00	6.77	6.95	5.31	8.36	5.37	7.45	77	6.46	0.477
12/21/71	6.20	6.93	6.15	5.97	7.42	4.62	7.00	84	6.33	0.348
12/28/71	5.30	7.31	4.65	5.43	7.55	3.99	5.36	91	5.66	0.497
1/4/72	5.66	6.58	5.74	5.17	9.07	8.72	5.12	98	6.58	0.626
1/5/72	4.69	7.11	6.99	5.59	9.14	5.54	6.95	99	6.57	0.552
1/6/72	4.94	8.27	6.39	5.08	8.66	6.43	5.91	100	6.53	0.548
1/7/72	4.83	7.42	6.06	4.39	6.80	5.20	6.79	101	5.93	0.432
1/8/72	4.74	8.36	6.87	5.33	9.21	6.40	8.82	102	7.10	0.659
1/9/72	5.40	8.79	6.14	5.06	7.89	5.88	5.69	103	6.41	0.525
1/10/72	6.03	7.38	8.39	5.61	7.01	5.31	7.93	104	6.81	0.448
1/13/72	5.35	9.34	6.42	6.18	7.61	6.45	5.95	107	6.76	0.502
1/18/72	6.03	8.45	6.60	7.13	7.61	5.48	7.10	112	6.93	0.368

APPENDIX C

MILK PRODUCTION

APPENDIX C

MILK PRODUCTION
Long Term Thyroprotein Feeding (Group A)

	Cow #							
Time Period	832	935	939	1008	1010	1034	1093	Mean
	(Milk lb/day)							
8/9/71-8/22/71	60.4	68.3	61.1	51.0	43.8	51.5	43.0	54.2
8/23/71-9/5/71	53.7	68.6	57.1	47.7	44.4	48.6	42.1	51.7
9/6/71-9/19/71	43.1	62.7	52.1	43.8	41.3	46.4	40.4	47.1
9/20/71-10/3/71	46.3	61.6	51.0	40.6	36.3	45.6	44.0	46.5
10/4/71-10/17/71	47.0	62.1	51.6	41.3	37.3	47.1	45.9	47.5
10/18/71-10/31/71	47.0	61.9	41.8	40.1	34.6	45.6	39.9	44.4
11/1/71-11/8/71	44.8	60.6	48.8	35.3	32.7	47.9	45.8	45.1
11/9/71-11/22/71	42.9	58.0	41.0	32.8	31.6	41.8	45.4	41.9
11/23/71-12/6/71	37.0	52.3	35.6	23.7	26.9	32.9	36.9	35.0
12/7/71-12/20/71	37.9	44.0	34.0	23.2	24.3	26.4	38.6	32.6
12/21/71-1/3/72	35.6	47.4	31.5	11.5	21.9	22.5	35.6	29.4
1/4/72-1/17/72	29.0	36.3	24.9	8.5	16.7	13.6	29.1	22.6

MILK PRODUCTION--Continued
Short Term Thyroprotein Feeding (Group B)

	Cow #							
Time Period	906	958	1037	1061	1072	1074	1087	Mean
				(Milk lb/day)				
8/9/71-8/22/71	45.1	55.4	72.5	49.1	51.8	44.3	41.9	51.4
8/23/71-9/5/71	42.4	51.6	68.1	48.6	47.3	42.9	43.1	49.1
9/6/71-9/19/71	38.9	50.1	61.0	47.0	42.5	37.8	43.1	45.8
9/20/71-10/3/71	40.2	48.0	60.6	47.5	41.5	37.7	40.3	45.1
10/4/71-10/17/71	44.5	47.6	63.2	50.3	45.4	38.4	41.0	47.2
10/18/71-10/31/71	46.2	44.1	60.6	45.5	46.5	32.5	42.8	45.5
11/1/71-11/8/71	47.1	41.1	55.1	48.8	45.4	37.1	40.1	45.0
11/9/71-11/22/71	41.0	34.8	49.8	39.0	37.0	35.1	34.3	28.7
11/23/71-12/6/71	33.4	29.8	42.0	40.5	31.7	30.1	35.4	34.7
12/7/71-12/20/71	sold	25.2	42.7	40.1	33.4	30.2	35.0	34.4
12/21/71-1/3/72	--	23.9	38.8	37.6	31.5	21.4	32.5	31.0
1/4/72-1/17/72	--	20.7	37.0	37.9	29.3	18.5	32.5	29.3

MILK PRODUCTION--Continued
Control (Group C)

Time Period	833	873	1073	1075	1078	1082	1091	Mean
			Cow #					
				(Milk lb/day)				
8/9/71-8/22/71	54.4	62.7	48.7	57.8	41.7	49.7	50.5	52.2
8/23/71-9/5/71	58.3	55.8	44.9	59.6	40.0	--	52.4	51.8
9/6/71-9/19/71	58.9	47.1	38.5	52.9	37.0	--	48.2	42.1
9/20/71-10/3/71	59.6	38.2	40.2	52.5	36.0	--	47.1	45.6
10/4/71-10/17/71	61.3	32.0	40.2	49.7	33.1	38.9	43.9	42.7
10/18/71-10/31/71	54.9	26.2	37.5	50.0	27.0	39.8	33.2	38.4
11/1/71-11/8/71	56.9	26.3	40.3	49.9	41.6	27.7	42.2	40.7
11/9/71-11/22/71	52.3	20.4	37.5	48.2	42.1	25.1	38.9	37.8
11/23/71-12/6/71	47.8	14.9	38.3	43.5	18.9	28.8	39.6	33.1
12/7/71-12/20/71	45.3	12.1	38.1	48.7	17.5	41.0	41.3	34.9
12/21/71-1/3/72	46.2	9.0	33.1	47.0	12.6	32.5	41.7	31.7
1/4/72-1/17/72	42.7	7.6	30.0	46.8	10.0	35.1	41.3	30.5