December 1, 1951

To the Graduate Council:

I am submitting to you a thesis written by Jesse Payton Boatman entitled "Effect of Thiouracil Produced Hypothyroidism upon the Semen and Testes of Dairy Bulls." 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.

L. E. Wylie
Major Professor

We have read this thesis
and recommend its acceptance:

Eric W. Swanson
Harold J. Smith
R. M. Lush

Accepted for the Council

E. A. Wilcox
Dean of the Graduate School

EFFECT OF THIOURACIL PRODUCED HYPOTHYROIDISM ON SEMEN AND TESTES OF DAIRY BULLS

A THESIS

Submitted to
The Graduate Council
of
The University of Tennessee
in
Partial Fulfillment of the Requirements
for the Degree of
Master of Science

---

by

Jesse Payton Boatman

December 1951

ACKNOWLEDGMENTS

The writer wishes to acknowledge the aid and direction of Dr. Eric W. Swanson, Associate Professor of Dairying, University of Tennessee; the assistance of Joe Bearden, former Instructor in Dairying; the assistance of Ben Throop, Laboratory Technician; the assistance of Dr. J. O. Andes, Plant Pathologist, University Agricultural Experiment Station, Knoxville, Tennessee; and the generous contribution of thiouracil by Lederle Laboratories.

TABLE OF CONTENTS

PAGE

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

For the past two decades the relationship of the thyroid glands to the genital system of the male has been a problem of interest to many investigators (4, 9, 24). It has been reported that one cause of reduced fertility in dairy bulls is an underactive thyroid with consequent reduced thyroxine secretion and lowered matabolism and seminal changes (9, 13, 24, 36, 40, 41). Feeding thyroxine has been advocated as a means of improving fertility in bulls (13, 40). On the other hand, thyroidectomized bulls have been reported to have normal semen (36). The essential role of thyroxine has been demonstrated definitely in rams by producing hypothyroidism by thiouracil feeding (11). Because of the similarity of seminal characteristics of rams and bulls, it was of interest to investigate the response of dairy bulls to such artificially induced hypothyroidism.

This investigation of the relation of the thyroid to reproductive physiology in dairy bulls was begun in The University of Tennessee Department of Dairying in the latter part of 1950. The phase of this investigation reported here deals with the attempts to feed dairy bulls methyl-thiouracil at a level necessary to produce mild hypothyroidism, and to observe the effect upon libido, seminal characteristics and glandular tissue changes as compared to that of normal bulls.

REVIEW OF LITERATURE

Absorption and elimination of thiouracil. Thiouracil is absorbed from the gastro-intestinal tract and taken to all parts of the body, tissues and fluids (52), with the greatest amount being stored in the pituitary, thyroid, adrenals and bone marrow. Thiouracil in the blood is largely bound to the protein with cells possessing several times as much as the plasma; it is absorbed rapidly from the digestive tract and eliminated quickly mainly by way of the kidneys (18).

Significant levels of thiouracil were maintained over a 22-hour period in 125 calves by oral administration of 2.0 grams at 12-hour intervals (18). The peak of concentration in the blood was reached at 4 to 8 hours when administered orally and in 2 to 4 hours when administered subcutaneously.

In cockerels (8) receiving 50 milligrams of radioactive thiouracil per os, 60 to 70 percent of the hormone was excreted within 24 hours. After this lapse of time the thyroid, the base of the feather shafts, and probably also the hypophysis, were the only tissues which retained appreciable amounts of the radioactive substance. After 96 hours only traces of methyl-thiouracil could be detected in the carcasses (8). When the 50 milligram dose was repeated after 24 hours, a cumulative effect was observed (8).

Most of the tissues of the body and also gastro-intestinal contents possess the capacity to destroy thiouracil (52). One third of the ingested drug is excreted as such in the urine, but none in the feces (52).

Rawson et al. (37a) state that thiouracil stored in the organs possesses the ability to resist iodine, which is possibly one principal means by which thiouracil blocks the production of thyroxine.

Effect of thiouracil feeding on semen. The administration of a chemical in great enough quantities to produce hypoplasia or regression of tissues within the testes or other parts of the genital tract would very likely be detrimental to the normality of the semen. In experiments with cockerels, thiouracil treated birds were compared with normal birds under otherwise similar treatment, used as controls, and it was found that testes size and tubule diameter were much smaller than in the control group (3, 12, 42, 43, 46), and that regression of the tubules in the thiouracil treated group was in some cases associated with interruption of spermatogenesis (11, 12, 24, 45). When the fattening ration was replaced by a normal diet and thiouracil discontinued, spermatogenesis was resumed. Therefore, it is believed that regression of the testes is a secondary effect of changes in the thyroid glands, brought about either by excessive feeding coupled with restriction of movement or by the administration of methyl-thiouracil (12). Thiouracil feeding was found to decrease the motility, viability and longevity of the sperm in certain animals (16, 11, 24, 45, 46).

When hens were inseminated with semen from cocks of three groups: controls, those receiving 0.2 percent thiouracil, and those receiving 0.5 percent thiouracil, semen from controls was 94.7 percent fertile for 550 eggs, from the 0.2 percent group it was 85.4 percent fertile for 192 eggs, and the 0.5 percent group was 76.2 percent fertile for

420 eggs (46). The feeding of thiouracil to cocks has been found to decrease greatly the comb area.

Other evidences, however, indicate that the feeding of thiouracil has no significant effect upon semen volume, motility or sperm concentration (22, 23, 24, 46). Still others have found the hatchability of eggs to be improved by feeding thiouracil (29).

Hypothyroidic conditions that caused a marked decrease in sperm numbers, motility and an increase in sperm abnormalities in rams had no noticeable effect upon the libido (11). The feeding of 0.25 percent thiouracil to rats for 14 days had no effect upon the size, weight or microscopic appearances of testes and ovaries (46). Of eleven males used, ten sired litters and it was concluded that hypothyroidism induced by thiouracil feeding had no effect upon the reproductive system of the male rats (22, 44, 46). Semen volume from the control group was insignificantly less than that from those groups receiving 0.2 percent and 0.5 percent thiouracil, though the motility and longevity of semen from groups getting 0.2 percent and 0.5 percent were significantly less than for the control (46).

Effect of chemical hypothyroidism and thyroidectomy on libido and spermatogenesis. Thyroidectomy in the bull leads to complete loss of libido (36, 47). This loss of libido does not affect spermatogenesis since ejaculates obtained by massage of the ampulla are normal in sperm activity, morphology, longevity and fertilizing ability (36). The administration of thyroxine immediately restores libido (13, 40).

Feeding thiouracil at a 0.75 grams per 100 pounds of body weight level completely stops the production of thyroxine in cattle, sheep and

goats (49), which is equivalent to total thyroidectomy in so far as thyroxine production is concerned.

Thyroidectomized fowl were found, on autopsy, to have regenerative tissue (46). One thyroidectomized calf showed rough coat and other symptoms of thyroxine deficiency at three weeks after the operation, but at seven weeks after operation the calf showed again normal vigor and a glossy coat (14). Many investigators have noted a pronounced effect of thyroidectomy upon comb and testes of birds (24). It was found that thyroidectomy would decrease the testes size by as much as 80 percent in eleven days and as much as 90 percent within twenty days in chicks and similarly in drakes (24). The size of the penis was affected more than the testes (24). Greenwood and Chu observed regression of testicular size and cessation of spermatogenesis in Brown Leghorns after thyroidectomy (19).

Though it is hard to reach agreement, a number of investigators hold the view that the thyroid has no direct effect on the testes, and that any reproductive disturbance in the male in hypothyroidism and hyperthyroidism is due, not primarily to endocrine imbalance, but to a changed metabolic status (41, 33); change in nervousness and nervous irritability (10); general growth disturbances (48); or change in the complex interrelations between the endocrine system and body metabolism as a whole (25, 39).

Thyroidectomized rams yielded a decrease in semen concentration and an increase in percentage of abnormal sperm (7). The testes were edematous; there was a decrease in the interstitial tissue and

sloughing and pycnosis in the seminiferous tubules (7).

Effect of thyroxine on semen. It has been noted that the addition of thyroxine to semen diluter produced a definite increase in conception rate (9). It is believed by several writers that thyroxine plays an important role in maintaining the normal pituitary-gonadal balance, evidenced by the reduction in the level of pituitary gonadotrophin in thyroidectomized goats (9, 10). There are indications that the addition of thyroxine influenced the metabolism of semen, and thyroxine added to critically concentrated bull semen caused an increase in oxygen consumption (41). As a result of a series of trials with various concentrations it was found that the optimum level was 0.3 micrograms per 10 milliliters of diluted semen to produce the greatest oxygen absorption rate (41).

Effect of thiouracil upon the thyroid gland. Thiouracil has been reported by some (46) to have an effect upon various tissues of the body, while others believe that it has a direct effect upon the thyroid (20, 21, 23, 43) and secondary effects upon the other organs. It is a very popular opinion though that the administration of thiouracil has either a direct or an indirect effect upon the thyroid of various animals (2, 3, 4, 6, 20, 28, 30, 46). The rat thyroid is greatly enlarged (22, 26, 46); broilers' thyroid is greatly enlarged with cysts (3, 21, 46); lambs' thyroid greatly enlarged (2, 4); and pigs' and hogs' thyroids are greatly enlarged, two to three times normal size. Feeding steers four grams of thiouracil per day enlarges the thyroid by 50 percent or more, but a 2-gram level produces no noticeable change (6). Some broilers fed at a 0.2 percent level had thyroids enlarged to 20 times normal

size and one other was 70 times normal size. From this evidence we would conclude that the administration of thiouracil at a 0.2 percent level or greater would produce thyroid enlargement.

Effect of thiouracil upon BMR. It is quite possible that the principal effect of thiouracil upon the male animal is the lowering of basal metabolism. Meyer and Ranson found that a 0.5 percent feeding of thiouracil lowered the BMR to -41 percent (32, 46). Reineke produced a -23.7 percent BMR by feeding a ration of 0.1 percent thiouracil (38). Furthermore, it has been found that the nervousness and irritability of the thiouracil-fed animals was due to a lowered basal metabolism (9, 30, 32, 34, 35). On the other hand, the administration of thiouracil does not have the same effect upon all animals, since light dosages tend to bring the metabolic rate back to normal in some disorders of the human (23).

Role of thyroid in sexual development in the male. A series of experiments (27) showed that hyperthyroidism aided spermatogenesis and increased secretory action of the interstitial cells of the testis for mice, rabbits and rams and that thyroidectomy and prolonged thiouracil treatment interfered with normal processes of spermatogenesis. The seminiferous tubules showed atrophic and degenerative changes, the spermatogonial cells being disorganized and the tubules appearing to contain an abnormal accumulation of cells.

Mild hyperthyroidism resulted in a precocious development in the young male rabbit and ram, while prolonged hypothyroidism or severe hyperthyroidism interfered with normal reproductive processes (27).

Continued administration of large doses of thiouracil to growing males markedly impaired the sex behavior and the semen was of very poor quality (27).

The depression of sexual development during hypothyroidism (27) may be due, in part, to reduced secretion of the pituitary gonadotrophins, while the increased functional activity in hyperthyroidism is probably due to increased output of hypophyseal gonadotrophic hormones which in the presence of thyroxine are responsible for growth of the male organs. The follicular stimulating hormone stimulates the growth of the seminiferous tubules, and the increased production of luteinizing hormone stimulates the secretion of the male sex hormone by the Leydig cells of the testes (27).

EXPERIMENTAL PROCEDURE

Plan of investigation. The plan of investigation was to feed thiouracil to two normal and sexually mature dairy bulls to produce a mild hypothyroidism, and to observe changes in reproductive activity measured by libido and semen quality. Thiouracil was to be fed twice daily throughout the feeding period. The quantity to be fed was five grams daily at the beginning with periodic increases as would be necessary to reach an effective feeding level, determined by the blood plasma content, and to continue at this level for a period deemed necessary to produce hypothyroidism.

Semen was to be collected each Thursday, examined, diluted and stored for further study and examination. Heart rate and libido would be determined at each collection period.

In the semen studies an initial motility rating was to be made and differential stains made for live-dead and morphology studies. The influence of thyroxine on semen from hypothyroid bulls, and the effect of thiouracil upon semen from normal bulls was to be determined.

At the end of the experimental period both treated bulls were to be slaughtered. Their thyroids and testes were to be sectioned and observed for any histological variations from normal.

The period of investigation was to be divided into three parts: a period of acclimation, a period of thiouracil feeding, and a post-thiouracil feeding period. The first part was an adjustment period in which the animals were to have their rations changed from a showing to a growing ration, their habitat from a show circuit to the confines of

a dry lot and stable, and to become acquainted with the breeding rack and the process of collecting semen artificially. The second part was the hypothyroid period and the third part was the leveling off and conclusion.

Experimental animals. Bulls used for semen collection were six sexually mature bulls in the University of Tennessee dairy herd. Two of these selected for thiouracil feeding were Jerseys, 22 months old at the beginning of the investigation. These are designated hereafter as R and C. Specimens of normal thyroid and testes were secured from a similar bull slaughtered at the East Tennessee Packing Plant, and designated as X.

Semen collections and examination. In the preparation for collection, the bulls were caught and tied one hour before time for collection. Then the heart rate was checked, by caudal pulse, before the teaser cow was brought about. At the time of collection each bull's libido was rated in figures, 0, 1, 2, 3, in which 0 indicates none and 3 is top rating. Collections were made once each week. Two ejaculates were taken at each collection when possible.

Semen was collected by means of an artificial vagina, constructed of a 14-inch rubber hose 2.25 inches in diameter with soft rubber inner liner and a soft rubber funnel supporting a graduated collection tube. The space between the tubing and inner liner was filled with water hot enough to maintain a temperature of 115°F to 120°F at time of collection. A flannel-lined sheath was put over the funnel and collection tube to protect the semen from temperature shock. Immediately after collection

the tubes of semen were placed in a vacuum bottle containing water at approximately 95°F to maintain a constant temperature until it reached the laboratory for examination, dilution and storage.

At the laboratory the semen was examined for initial motility rated as the percentage of progressively motile sperm. As soon as possible a differential stain was made for morphological examination and the determination of the percentage of live sperm. The differential stain consisted of 2 percent eosin and 5 percent nigrosine in a 3 percent $Na_3C_6H_5O_7.2H_2O$ solution (50). The semen was diluted at 1:25 in a diluter consisting of 1 part fresh egg yolk, free from white and membrane, to 3 parts of 3 percent sodium citrate dihydrate buffer solution. Streptomycin was added to all diluted semen at the rate of one milligram per milliliter. The diluted semen was gradually cooled in a paper-wrapped vial in a refrigerator, operating at 40°F, where it remained in storage over the entire period of observation.

Motility readings were made by placing a drop of mixed semen from each vial on a warm slide in a microscope stage incubator set at 100°F, covering with a cover glass and promptly estimating the percentage of progressively motile sperm to the nearest 5 percent. Semen retaining any motility was rated 10 days.

Differential stains were filed as they were made and examined later. Counts of dead sperm and abnormal sperm from examining not less than 300 sperm per slide were made and listed in tabular form. The recorded abnormalities were: proximal protoplasmic droplet (when the droplet appeared at the neck of the mid-piece), abnormal heads

(tapering heads, pyriform heads, small heads or enlarged heads), separate heads (in this case the heads and not the tails were counted), abnormal mid-piece (filiform, beaded mid-piece, enlarged mid-piece, or doubled mid-piece), abnormal tails (this included coiled tails of various types and double tails).

Thiouracil feeding and blood plasma analysis. Thiouracil was fed in the grain ration in two equal portions at night and morning feedings for all levels of thiouracil feedings. Each bull received three pounds of grain and six pounds of hay twice daily until thiouracil feeding reached its topmost level when the grain remained as before and the hay feeding was reduced to three pounds twice daily to force the consumption of grain and thiouracil. The feeding of thiouracil was increased after each analysis until a desired level of thiouracil in the blood was reached; this took place in the following manner: on February 4 thiouracil was started at 2.5 grams per bull twice daily, on May 4 the thiouracil ration was increased to 4 grams per bull twice daily, on May 9 thiouracil was increased to 7.5 grams per bull twice daily, on May 15 thiouracil was increased to 12.5 grams per bull twice daily, and on May 23 it was increased to 15 grams per bull twice daily, a level at which the analysis showed a constant plasma thiouracil content.

The blood for the analysis was taken from the jugular vein of each experimental bull and one normal bull at 9 A. M., 1 P. M. and 5 P. M. on May 1, 1951. The normal bull plasma was used as control with which to get a zero reading on the instrument.

Potassium oxalate was used in the collection tubes to prevent the clotting of blood. The tubes of blood were stored over night in the

refrigerator to facilitate separation of the cells and plasma when they were centrifuged.

Blood plasma thiouracil was determined by an improved method based on the use of Grote's Reagent (35). 1.0 milligram percent and 2.5 milligram percent solutions of thiouracil were used as standards for measuring the thiouracil content of the plasma in the analytical reading.

Influence of thyroxine upon sperm livability. Since thiouracil blocks the production of thyroxine (26), this condition may possibly be injurious to the sperm livability. A total of 91 samples of semen from the hypothyroid bulls were treated with 0.1 microgram thyroxine per milliliter of diluted semen and compared with the same ejaculates diluted without thyroxine. All samples were treated in the same manner in storage and examination.

Influence of thiouracil upon sperm livability. Thiouracil content of the blood and gland secretions may also be detrimental to the motility and livability of the sperm. To investigate this possibility, semen from normal bulls was taken and diluted at the same rate as the semen samples from the hypothyroid bulls. Antibiotics were used in the same proportions and thiouracil added in various concentrations from 0 to 100 milligram percent. All semen samples were refrigerated at 40°F and rated for motility as described above.

Histological examination of thyroid and testes. The bulls, R and C, were slaughtered on July 19, 1951, and sample tissues were taken from the thyroid and testes of each bull for histological examinations.

These tissues were preserved in Bouin's fixative (5), and mounted in paraffin blocks for sectioning. The sections were cut 5 microns in thickness, floated on water and picked up on slides coated with Mayer's albumin. They were stained with hematoxyline and counter stained with 0.25 percent alcoholic eosin solution. The sections were mounted in Canada balsam under glass cover plates. Representative fields of the sections were photomicrographed with an enlargement of 150X. Histological examination was also made of each section by careful observation at 450X and 930X magnification. Tissues from a normal dairy bull of about the same age as the hypothyroid bulls, R and C, were taken from a bull at the slaughter house and fixed, sectioned and examined in the same manner as that of the experimental bulls.

RESULTS

Physical appearance and behavior. Bulls R and C were indolent and had little tendency to want to play and exercise; there was also a noticeable rough coat in the spring. There were no symptoms of nervousness and irritability at any time. However, when they were out in their respective lots and other animals were brought about, they failed to show interest in them as did the normal bulls. They were not frequently seen pawing the ground and lowing when other animals or people came near, and would often remain at the back side of their lots until they were driven into the barn. When disturbed or excited the bulls appeared to be very alert and to have a lot of energy as is shown in Figures 1 and 2. These photographs were taken two months after the starting of the thiouracil feeding at the level of 5 grams per bull per day and one month before the higher level of feeding was started.

Semen production and sperm viability. During the period of acclimation the collection from the two experimental bulls was very satisfactory. Libido was usually very good and the volume, concentration and physical appearance were nothing short of normal. Tables I and II show sums and averages for the four periods considered. These periods were: pre-experimental, 90-day thiouracil feeding at 5 gram level, the period of thiouracil feeding at the increased levels, and post-thiouracil feeding period. The changes noted in the studies of semen of R were a decrease in volume in the post thiouracil period, and a progressive increase in initial motility during the trial. The studies of semen from C showed

Figure 1

Photograph of Bull R Taken Two Months after Thiouracil Feeding Was Started

Figure 2

Photograph of Bull C Taken Two Months after Thiouracil Feeding Was Started

TABLE I

PERIOD AVERAGES OF VOLUME, CONCENTRATION AND MOTILITY OF SEMEN FROM BULL R

Period	Thio-uracil Feeding Level	Ejaculation Volume	Concentration $1000mm^3$	Number of Samples	Motility and Hours of Storage										
					0	24	48	72	96	120	144	168	192	216	240
	g.														
12-28-50 to 2- 4-51	0	2.0	1748	14	65	59	51	42	35	29	24	18	14	11	9
2- 4-51 to 5- 4-51	5	1.7	1546	25	70	62	55	46	35	27	21	16	12	9	6
5- 4-51 to 6-15-51	30	1.8	1751	11	73	68	58	51	44	31	26	20	15	9	7
6-15-51 to 7-19-51	0	2.9	1404	10	76	68	57	46	37	30	20	12	6	3	2

TABLE II

PERIOD AVERAGES OF VOLUME, CONCENTRATION AND MOTILITY OF SEMEN FROM BULL C

Period	Thio-uracil Feeding Level	Ejacu-lation Volume	Concen-tration $1000mm^3$	Number of Samples	Motility and Hours of Storage										
					0	24	48	72	96	120	144	168	192	216	240
	g.														
12-28-50 to 2- 4-51	0	3.0	1172	11	65	57	49	41	35	29	25	20	16	13	10
2- 4-51 to 5- 4-51	5	2.6	926	26	64	55	49	40	33	28	24	18	15	12	9
5- 4-51 to 6-15-51	30	3.0	990	12	67	59	54	48	42	30	28	22	17	12	10
6-15-51 to 7-19-51	0	2.9	1120	9	75	66	54	45	37	30	21	16	13	7	6

a decrease in concentration during the thiouracil period and a slight increase for the post-thiouracil period, with a slight increase in initial motility in the last two periods.

Morphology of spermatozoa. At the first of the period of acclimation and training the semen was of low volume, slightly low in concentration and had a relatively high percentage of dead and abnormal spermatozoa. This period was short, though it did affect the average somewhat. Tables III and IV show the percentage dead and the various abnormalities for both bulls R and C. These ratings are averages of the entire period as indicated in each table. All abnormalities and dead sperm in the semen of bull R of Table III showed a decrease in percentages in each of the thiouracil feedings and an increase in all percentages of abnormalities in the post-thiouracil feeding except that of abnormal tails. For bull C, in Table IV, the percentages were in decreasing order for thiouracil periods and increasing in post-thiouracil, except for separate heads, protoplasmic droplet and abnormal mid-piece.

Heart rate and libido. The bulls' libido was normal throughout the feeding period. However, it was noted at at times when the libido rated low a change of the teaser from cow to bull caused the bulls to serve the artificial vagina readily. During the training period R was very slightly hurt on the breeding rack which caused him to show symptoms of a low rating libido, but when the teaser was taken out of the rack, he served the artificial vagina readily. Even during this period of shyness the semen volume was normal, which was in accordance

TABLE III

LEVEL OF THIOURACIL FEEDING, NUMBER OF SAMPLES, DEAD SPERM, ABNORMAL HEADS, SEPARATE HEADS, PROXIMAL DROPLET, ABNORMAL MID-PIECE, ABNORMAL TAILS, AND TOTAL ABNORMALITIES FOR BULL R

Period	Level of Thiouracil Feeding	Number of Samples	Dead Sperm	Abnormal Heads	Separate Heads	Proximal Droplet	Abnormal Mid-piece	Abnormal Tail	Total Abnormalities
	g.		%	%	%	%	%	%	%
12-28-50 to 2- 4-51	0	9	24.7	0.79	1.89	0.35	1.67	2.74	7.44
2- 4-51 to 5- 4-51	5	21	18.1	0.78	1.19	0.24	1.59	1.37	4.88
5- 4-51 to 6-15-51	30	11	13.9	1.34	0.82	0.18	0.67	1.16	4.58
6-15-51 to 7-19-51	0	8	14.3	2.65	0.97	0.58	2.62	0.90	7.54

TABLE IV

LEVEL OF THIOURACIL FEEDING, NUMBER OF SAMPLES, DEAD SPERM, ABNORMAL HEADS, SEPARATE HEADS, PROXIMAL DROPLET, ABNORMAL MID-PIECE, ABNORMAL TAILS, AND TOTAL ABNORMALITIES FOR BULL C

Period	Level of Thiouracil Feeding	Number of Samples	Dead Sperm	Abnormal Heads	Separate Heads	Proximal Droplet	Abnormal Mid-piece	Abnormal Tail	Total Abnormalities
	g.		%	%	%	%	%	%	%
12-28-50 to 2- 4-51	0	10	23.2	1.80	1.58	0.16	1.23	4.73	9.52
2- 4-51 to 5- 4-51	5	24	15.6	1.12	1.87	0.19	1.55	4.70	9.52
5- 4-51 to 6-15-51	30	12	13.4	1.22	1.81	0.19	1.00	3.85	8.04
6-15-51 to 7-19-51	0	7	15.3	3.81	1.16	0.60	2.89	1.32	9.74

with the findings in literature (15).

The heart rate and libido were not noticeably affected by the thiouracil feeding. Aside from the expected variation due to individual differences, these rates were normal as is indicated in the following tables. Tables V and VI show thiouracil feeding level, number of semen collections per period per animal, the average libido rating and the average heart rate count.

Blood plasma and thiouracil. On the day following blood samplings the analysis for thiouracil content was made. Tables VII and VIII show the day of the samplings, the level of thiouracil feeding up to that time, and the thiouracil level in the blood at that level of feeding. A constant level of blood thiouracil was not found until 15 grams were fed twice daily. Failure to find thiouracil in measurable quantities on May 22 when 25 grams per day were being fed was probably due to poor consumption of the feed in which the thiouracil was mixed. This was corrected before the May 28 sampling by reducing the hay allotment. It was further noted that the thiouracil findings in the blood plasma at the lower levels of feeding were irregular. This was possibly due to piece-meal eating of grain throughout the day.

Effect of thyroxine on livability of sperm of thiouracil treated bulls. Table IX shows the comparative 10-day average reading of motility for the entire thiouracil feeding period. The findings in this experiment show no significant advantages in adding thyroxine at the rate of 0.1 microgram per milliliter of diluted semen.

TABLE V

NUMBER OF COLLECTIONS, THIOURACIL FEEDING LEVEL, AVERAGE LIBIDO AND HEART RATE FOR BULL R

Period	Number of Collections	Thiouracil Feeding Level	Average Libido	Average Heart Rate
		g.		
11-28-50 to 2- 4-51	14	0	3.0	67
2- 4-51 to 5- 4-51	25	5	2.0	65
5- 4-51 to 6-15-51	11	30	2.5	64
6-15-51 to 7-19-51	10	0	2.8	62

TABLE VI

NUMBER OF COLLECTIONS, THIOURACIL FEEDING LEVEL, AVERAGE LIBIDO AND HEART RATE FOR BULL C

Period	Number of Collections	Thiouracil Feeding Level g.	Average Libido	Average Heart Rate
11-28-50 to 2- 4-51	11	0	3	63
2- 4-51 to 5- 4-51	26	5	3	62
5- 4-51 to 6-15-51	12	30	3	62
6-15-51 to 7-19-51	9	0	3	60

TABLE VII

THIOURACIL FEEDING LEVEL AND BLOOD PLASMA THIOURACIL CONTENT OF BULL R

Period	Thiouracil Feeding	Thiouracil in Blood 9:00 A. M.	Thiouracil in Blood 1:00 P. M.	Thiouracil in Blood 5:00 P. M.
	g.--Day	mg.--%	mg.--%	mg.--%
Average of 3 consecutive analyses on May 1, 2, 3	5	0.05	0.017	0.00
May 8	8	0.10	0.000	0.00
May 14	15	0.05	Trace	0.10
May 22	25	0.00	0.000	0.10
May 28	30	0.75	0.350	0.20

TABLE VIII

THIOURACIL FEEDING LEVEL AND BLOOD PLASMA THIOURACIL CONTENT OF BULL C

Period	Thiouracil Feeding	Thiouracil in Blood 9:00 A. M.	Thiouracil in Blood 1:00 P. M.	Thiouracil in Blood 5:00 P. M.
	g.--Day	mg.--%	mg.--%	mg.--%
Average of 3 consecutive analyses on May 1, 2, 3	5	0.03	0.00	0.02
May 8	8	0.05	0.00	0.00
May 14	15	0.00	0.00	0.00
May 22	25	0.00	Trace	0.00
May 28	30	0.15	0.20	0.20

TABLE IX

MOTILITY OF THYROXINE TREATED SEMEN AS COMPARED TO UNTREATED SEMEN

Animal	Semen Treatment	Motility and Hours of Storage										
		0	24	48	72	96	120	144	168	192	216	240
R	None	73	66	57	48	38	32	25	20	15	11	9
R	Thyroxine	73	65	57	48	39	29	23	17	11	7	5
C	None	68	60	52	44	37	29	24	18	15	10	8
C	Thyroxine	68	60	53	44	38	30	24	19	15	11	8

Effect of thiouracil on livability of sperm of normal bulls. Table X shows the average 10-day readings for 19 to 23 samples at each level of thiouracil from 0 to 100 milligram percent. With the exception of some unknown cause for the 2 and 5 milligram percent treated samples dropping down on two different occasions there seemed to be no difference in the treated and untreated semen in motility and livability.

Histological observations. On July 19, 1951, the bulls (R and C) were slaughtered, and thyroid glands, testes and genital tract were disected for examination. Photomicrographs of sections of the thyroid and testes of R, C and X (normal bull) are shown; in Figures 3, 4 and 5 respectively are thyroid, and 6, 7 and 8 respectively are testis.

The weight of the normal dairy bull thyroid has been reported about 32.5 grams (14). The weights of the treated bulls' thyroids at the time of slaughter were 54 grams for R and 59.4 grams for C. Thiouracil feeding had been discontinued 35 days before the bulls were slaughtered. Therefore, these weights probably do not represent the peak increase in thyroid size due to thiouracil feeding. The size increase was largely due to accumulation of colloid. In Figures 3 and 4 are shown quite a contrast in the sizes of the follicles from the treated bulls and the sizes of the follicles in Figure 5 from the normal bull. The follicular cross-sectional area as indicated in Figures 3 and 4 is from two to three times as great as that of Figure 5. Also the height of the epithelial cells in each of the Figures 3 and 4 is about one third that of Figure 5. These conditions, colloid accumulation and inactive epithelium, indicate that definite hypothyroidism had been produced in these bulls.

TABLE X

LIVABILITY AND MOTILITY OF THIOURACIL TREATED SEMEN

Thiouracil Concentration in mg.--%	Number of Treated Samples	Motility and Hours of Storage										
		0	24	48	72	96	120	144	168	192	216	240
Control	23	76	70	57	47	38	32	25	20	15	11	9
0.1	23	76	69	55	47	38	32	25	19	15	11	7
0.3	23	76	69	57	47	39	34	27	22	17	13	9
0.6	23	76	68	55	44	35	29	22	16	11	7	4
1.0	23	76	69	57	45	36	29	23	16	10	6	4
2.0	23	76	68	55	44	34	27	21	14	9	5	3
5.0	23	76	67	50	34	21	14	10	5	2	1	.3
10.0	23	76	68	53	43	31	26	20	15	10	8	5
50.0	19	76	67	54	42	31	26	21	19	14	12	9
100.0	19	75	67	55	44	33	29	24	19	15	14	9

In comparing the histology of testes in Figures 6 and 7 from the treated bulls, with that of Figure 8, no noticeable differences were apparent. The structure and arrangement of cells in the seminiferous tubules and the interstitial cells in testes from the treated and normal bulls are very much the same and appear to be quite normal. The columned appearances of the Sertoli cells are very much the same in Figures 6 and 7 as they are in Figure 8. The spermatids are forming normally in each case. Numerous spermatazoa were seen in all testes sections of all three bulls.

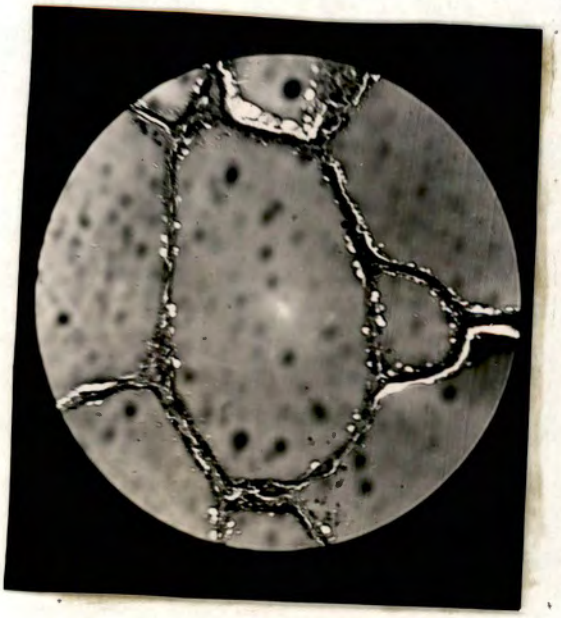

Figure 3

Photomicrograph of a Thyroid
Section of R, 150X Enlargement

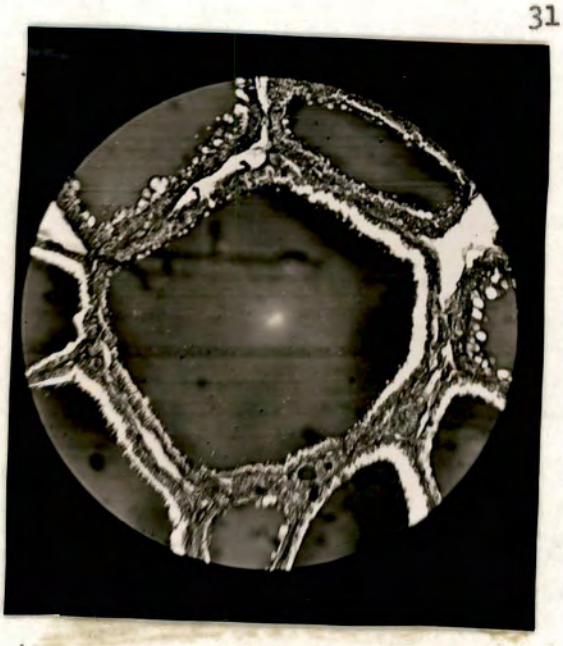

Figure 4

Photomicrograph of a Thyroid
Section of C, 150X Enlargement

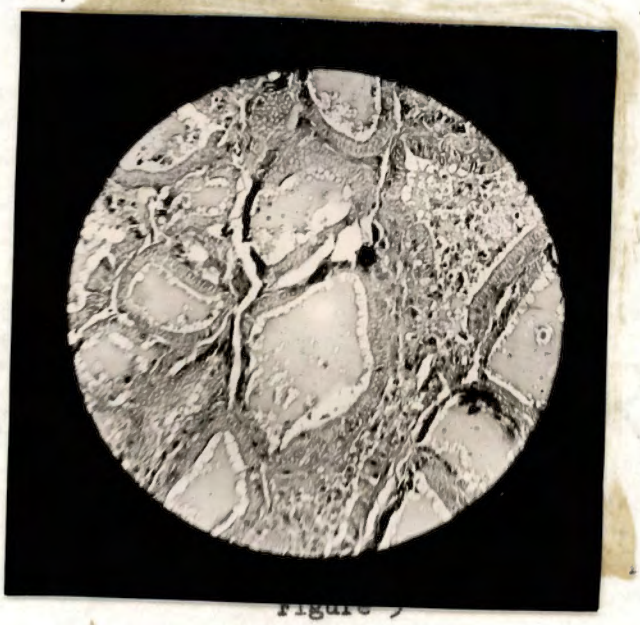

Figure 5

Photomicrograph of a Thyroid
Section of X, 150X Enlargement

Figure 6

Photomicrograph of Section of
Testis of R, 150X Enlargement

Figure 7

Photomicrograph of Section of
Testis of C, 150X Enlargement

Figure 8

Photomicrograph of Section of
Testis of X, 150X Enlargement

DISCUSSION

These bulls showed no hesitancy in eating their grain containing thiouracil until the level of 25 grams was arrived at. At this time a new palatable hay took the place of the old hay previously fed, and might have made the grain and thiouracil relatively less tasty. Previous investigators (6), however, have found difficulty in getting cattle (steers) to eat grain containing thiouracil in quantities of 6 grams or greater. By reducing the hay ration for R and C to half ration they were easily influenced to eat all their grain containing as much as 30 grams of thiouracil per 6 grams of grain.

This investigation has shown that thiouracil can be fed to sexually mature dairy bulls at the following levels as percent of the grain rations for the periods indicated without producing noticeably harmful effects: 0.19 percent for 90 days, 0.29 percent level for 6 days, 0.55 percent for 7 days, 0.91 percent for 7 days, and 1.1 percent level for 17 days. The last amount was found to be necessary in order to maintain constant plasma levels of thiouracil. Previous investigators (6) had found that hypothyroidism in cattle (steers) could be produced by feeding 0.20 percent or more thiouracil in the grain ration. It is highly possible that hypothyroidism was produced in these bulls before a constant readable level was obtained in the blood. The histological appearance of thyroids of the treated bulls indicated that hypothyroidism had been produced in this trial. The physical appearance and actions of the bulls also was indicative of hypothyroidism.

There were no consistent harmful effects of thiouracil feeding upon the volume, appearance and initial motility of semen collected from

these bulls every seventh day. The average initial motility of the semen for the thiouracil period was 74 percent progressively motile sperm for bull R and 71 percent for bull C. One cow was bred with three-day-old semen (taken during the thiouracil feeding period) from R, and conceived on first service. These seminal characteristics are in agreement with observations on thyroidectomized bulls (36).

It has been reported (40) that thyroxine added to semen improves the respiration and consequently improves the motility and fertilizing ability of the semen of some bulls. It is not clear whether or not such improvement is due to supplementing semen which is from thyroxine-deficient bulls. Since the bulls in this trial could be considered thyroxine deficient, if this situation influenced the response to thyroxine, improvement should have occurred. In this experiment thyroxine was added to diluted semen at the rate of 0.1 microgram per milliliter without any significant effect.

Thiouracil added to diluted semen from normal bulls in varying concentrations had no significant effect. In this experiment it was found that neither the presence of thiouracil nor the absence of thyroxine in minute quantities had any effect upon motility or livability of bull semen.

Thyroprotein has been reported to aid in the fertility of dairy bulls (17, 33, 37), since it carries an added amount of thyroxine; so we might expect thiouracil to hinder their fertility because of its thyroxine blocking ability (26, 37a). The findings here did not indicate any variation in the histology of tissues of the semen producing

organs of dairy bulls as a result of thiouracil produced hypothyroidism. It appears from the results of this and other studies that benefits derived from thyroxine addition to dairy bull rations must be primarily through an increase in general metabolism rather than through influencing the activity of the genital system. It must be assumed, therefore, that thyroxine would be of no value in the treatment of bulls with marked seminal changes. However, there is a possibility that the results secured in practice by feeding thyroxine to old bulls (37) cannot be adequately explained from these experiments with young bulls. The role of thyroxine in reproductive physiology may be influenced in some manner by the age of the bull. Further studies of known hypothyroid old bulls are needed to clarify this point.

CONCLUSION

The feeding of thiouracil to two sexually mature dairy bulls at a level of 5 grams to 30 grams per bull per day (0.19 percent to 1.1 percent of grain ration) over a period of 17 weeks had no harmful effect upon the testes. It did not lower the initial motility or the livability of the semen. It did not increase the average percentage of dead sperm and various abnormalities of bull sperm. It did not destroy the bulls' interest in the estrus cow, although there was indication of general decreased alertness.

Sections of the thyroid glands showed that thyroid physiology had been altered by the treatment. It must be assumed that some degree of hypothyroidism was developed. Apparently, normal thyroxine secretion is not essential to satisfactory functions of the testes in the bovine male.

Semen of these hypothyroid bulls was not benefited by the addition of 0.1 microgram of thyroxine per milliliter of diluted semen. It was also demonstrated that the small amounts of thiouracil expected in the semen of the treated bulls would not be responsible for abnormal motility and livability of sperm in storage.

BIBLIOGRAPHY

BIBLIOGRAPHY

1. Anderson, J. Thyroidal Functions, Semen of Animals and Its Use for Artificial Insemination. Edinburgh: Imperial Bureau of Animal Breeding and Genetics, 1945. 151 pp, 28-29.

2. Andrews, F. N., Beeson, W. M., Barrick, E. R., and Harper, C. "The Influence of Thiouracil and Thiourea on the Growth and Fattening of Lambs," J. Anim. Sci., 6:3-11, 1947.

3. Andrews, F. N., and Schnetzler, E. E. "Influence of Thiouracil on Growth and Fattening in Broilers," J. Poult. Sci., 25:124, 1946.

4. Barrick, E. R., Beeson, W. M., Andrews, F. N., and Harper, G. "The Influence of Thiouracil, Propylthiouracil and Thyroproteins on the Growth and Fattening of Lambs," J. Anim. Sci., 8:243, 1949.

5. Becker, E. R., and Roudabush, R. L. Bouin's Fixative, Brief Directions in Histological Technique. Ames, Iowa: The Iowa State College Press, 1945. 80 pp, 13-14.

6. Beeson, W. M., Andrews, F. N., and Brown, P. T. "The Effect of Thiouracil on the Growth and Fattening of Yearling Steers," J. Anim. Sci., 6:16, 1947.

7. Berliner, V., and Warbritton, V. "The Pituitary and Thyroid in Relation to Sperm Production in Rams," Am. Soc Anim. Prod. Proc., 137:142, 1937.

8. Bezem, J. J., Lever, J., and Brumekreeft, F. "Absorption and Excretion of Orally-Administered Radio-Active Methyl-Thiouracil in Cocks," Anim. Breeding Abst. 19, No. 1, 104, 1951.

9. Blaxter, K. L., Reineke, E. P., Cromption, E. W., and Peterson, W. E. "The Role of Thyroidal Materials and of Synthetic Goitrogens in an Animal Production and an Appraisal of Their Practical Uses," J. Anim. Sci., 8:307-314, 1949.

10. Blaxter, K. L. "Severe Experimental Hypothyroidism in the Ruminant," J. Agri. Sci., 38:1-15, 1948.

11. Bogart, R., and Mayer, D. T., "Environmental Temperature and Thyroid Gland Involvement in Lower Fertility of Rams," Mo. Agri. Exp. Sta. Res. Bul., 402:30-39, 1946.

12. Bretsneider, L. H. "On a Retarding Effect of Methyl-Thiouracil on Spermatogenesis in the Cock," (Landouwk, Tydschr), 61:912-916, Anim. Breeding Abst. 19: No. 1, 112, 1951.

13. Brody, S., and Frankenback, R. F., "Age Changes in Size, Energy, Metabolism and Cardio-Respiratory Activities of Thyroidectomized Cattle," Mo. Agri. Exp. Sta. Res. Bul. 349:11, 1942.

14. Bullard, J. F., Andrews, F. N. "Experimental Alteration of Thyroid Functions in Cattle," J. Am. Vet. Med. Assoc., 102:376-381, 1942.

15. Collins, W. J., Bratton, R. W., and Henderson, C. R. "Relationship of Semen Production to Sexual Excitement of Dairy Bulls," J. Dairy Sci., 34:224-228, 1951.

16. Dietrich, W. C., and Beutner, R. "The Mechanism of Action of Thiouracil," Soc. Exp. Biol. and Med Proc., 57:35, 1944.

17. Eaton, O. N., Schatt, R. G., Simmons, V. L., and Frank, A. H., "The Effect of Feeding Thyroprotein on Semin Characteristics of Rams," J. Anim. Sci., 7:449-454, 1948.

18. Ely, R. E., Olson, K. J., and Reineke, E. P. "Absorption and Elimination of Thiouracil in Ruminants," J. Anim. Sci., 7:208, 1948.

19. Greenwood, A. W., and Chu, J. P. "On the Relation between Thyroid and Sex Gland Functioning in Brown Leghorn Fowl," Quart. J. Exp. Physiol., 29:111-119, 1939.

20. Hoffman, E. "Thyroxine Secretion Rate and Growth in the White Pekin Duck," Poult. Sci., 29:109-114, 1950.

21. Huston, T. M., and Wheelerm, R. S. "Effect of Synthetic Thyroprotine on Seasonal Variation in Volume and Concentration of Cock Semen," Poult. Sci., 28:262, 1949.

22. Jones, G. E. S., Delfs, F., and Foote, E. C. "The Effect of Hypothyroidism on Reproduction in the Rat," Endocrinology, 38:337-344, 1946.

23. Kline, E. A., Ensminger, M. E., Cunha, T. J., Heineman, W. W., and Ham, W. E. "Effect of Adding Drugs to the Ration of Fattening Cattle," J. Anim. Sci., 8:411-424, 1949.

24. Kumeron, J. D. S., and Turner, C. W. "Effect of Hypothyroidism and Hyperthyroidism on Spermatogenesis in Birds," Poult. Sci., 28:653-665, 1949.

25. Leathem, J. H. "Influence on Organ Weight of Rates as Related to Food Intake," Proc. Soc. Exp. Biol. and Med., 61:203-205, 1946.

26. Malkiel, Saul. "A Note on the Mode of Thiouracil Action," Endocrinology, 38:58, 1946.

27. Maqsood, M. "The Role of Thyroid in Sexual Development in the Male," Nature, 166:692, 1950.

28. Mazzoni, R. "Experiment on the Use of Methyl-Thiouracil for Fattening Pigs," (Rin. Zootec., 22:163-166) Anim.Breeding Abst. 18, No. 1, Abst. No. 166:62, 1951.

29. McCartney, H. A., and Shaffner, C. S. "The Influence of Altered Metabolism upon Fertility and Hatchability in the Female Fowl," Poult. Sci., 29:67, 1950.

30. McMillen, W. N., Reineke, E. P., and Bratzler, L. J. "Feeding Thiouracil to Fattening Pigs," J. Anim. Sci., 5:419, 1946.

31. McMillen, W. N., Reineke, E. P., Bratzler, L. J., and Francis, M. J. "The Effect of Thiouracil on Efficiency of Gains and Carcass Quality in Swine," J. Anim. Sci., 6:305, 1947.

32. Meyer, A. E., and Ranson, G. V. "The Metabolism of Rats after Thyroidectomy or during Thiouracil Treatment and the Effect of Thyroid Feeding," Endocrinology, 36:259-265, 1945.

33. Moore, L. A. "Some Physiological Effects of Feeding Thyroprotein to Dairy Cows," J. Dairy Sci., 29:22, 1946.

34. Moring, R. E., and Shaffner, C. S. "A Thiouracil-Thyroprotein Treatment for Fattening Poultry," Poult. Sci., 28:504, 1949.

35. Olson, K. J., Ely, R. E., and Reineke, E. P. "An Improved Method for the Determination of Thiouracil," J. Biol. Chem., 169:681, 1947.

36. Peterson, W. E., Spielman, A., Pomeroy, B. S., and Boyd, W. L. "Effect of Thyroidectomy upon Sexual Behavior of the Male Bovine," Proc. Soc. Exp. Biol. and Med., 46:16, 1941.

37. Engle, E. T., editor. The Problem of Sterility. Reineke, E. P. "The Effect of Synthetic Thyroprotein on Sterility in Bulls," Princeton, N. J.: Princeton University Press, 1946. 251 pp, 233-242.

37a. Rawson, R. W., Tannheimer, J. F., and Peacock, W. "The Uptake of Radioactive Iodine by the Thyroids of Rates Made Goiterous by Potassium Thiocyanate and by Thiouracil," Endocrinology, 34:245-253, 1944.

38. Reineke, E. P., Mixner, J. P., and Turner, C. W., "Effect of Graded Dosages of Thyroxine on Metabolism and Thyroid Weights of Rats Treated with Thiouracil," Endocrinology, 36:66-67, 1945.

39. Schneider, B. A. "Effect of Feeding Thyroid Substances," Quart. Rev. Biol., 14:289-310, 431-450, 1939.

40. Schultze, A. B., and Davis, H. P. "Some Effects of Adding Thyroxine to Bovine Semen," J. Dairy Sci., 30:543, 1949.

41. Schultze, A. B., and Davis, H. P., "Effects of Thyroxine on Oxygen Consumption of Bovine Spermatozoa and Semen," J. Dairy Sci., 31:946, 1948.

42. Schultze, A. B., Hathaway, I. L., and Laeffel, W. J. "The Influence of Feeding Thiouracil to Dairy Bull Calves," J. Anim. Sci., 9:571-577, 1950.

43. Schultze, A. B., and Turner, C. W., "The Determination of the Rate of Thyroxine Secretion by Certain Domestic Animals," Mo. Agri. Exp. Sta. Res. Bul. 392, 1945.

44. Seegar, G. E., Jones, E. D., and Foote, E. C. "The Effect of Thiouracil Produced Hypothyroidism of Respiration in the Rat," Endocrinology, 38:337, 1946.

45. Shaffner, C. S. "The Influence of Thyroprotein Feeding on Semen Quality," Poult. Sci., 27:527-528, 1948.

46. Shaffner, C. S., and Andrews, F. N. "The Influence of Thiouracil on Semen Quality in the Fowl," Poult. Sci., 27:92-102, 1948.

47. Spielman, A. A., Peterson, W. E., Fitch, J. B., and Pomeroy, B. S. "General Appearance, Growth, and Reproduction of Thyroidectomized Bovine," J. Dairy Sci., 28:329, 1945.

48. Terrill, S. W., Krider, J. L., Carroll, W. E., and Hamilton, T. S. "Effect of Thiouracil on Rate and Efficiency of Gains of Swine," J. Anim. Sci., 8:501-507, 1941.

49. Turner, C. W., Reineke, E. P., and Schultze, A. B. "The Use of Thiourea in the Fattening of Meat Animals," J. Anim. Sci., 3:448, 1944.

50. Swanson, Eric W., and Bearden, H. J. "An Easin-Nigrosin Stain for Differentiating Live and Dead Bovine Spermatozoa," J. Anim. Sci., 10:981-987, 1951.

51. Vander Noot, G. W., Reece, R. P., and Shelly, W. C. "The Effect of Thiouracil in the Ration of Growing Swine," J. Anim. Sci., 6:12-15, 1947.

52. Williams R. H., and Clute, H. M. "The Absorption, Distribution, and Excretion of Thiouracil," Endocrinology, 35:201, 1944.