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# Effect of Thiamine, Riboflavin, Pantothenic Acid, and Niacin in Bovine Blood on the Economy of the Short-nosed Cattle Louse Haematopinus eurysternus (Nitzsch)

John Roger Crouch

# EFFECT OF THIAMINE, RIBOFLAVIN, PANTOTHENIC ACID, AND NIACIN IN BOVINE BLOOD ON THE ECONOMY OF THE SHORT-NOSED CATTLE LOUSE, HAEMATOPINUS EURYSTERNUS (NITZSCH)

BY

JOHN ROGER CROUCH

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This thesis is approved as a creditable and independent investigation by a candidate for the degree, Master of Science, and is acceptable as meeting the thesis requirements for this degree, but without implying that the conclusions reached by the candidate are necessarily the conclusions of the major department.

Thesis Adviser — Date

For - Head, Entomology-Zoology Department — Date

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# INTRODUCTION

A livestock producer will usually experience a louse infestation among his cattle sometime during his career. Most of the cattle that become louse-infested are easily ridded of their lice. There may be, however, one animal in a herd which is particularly susceptible to the lice and becomes chronically infested. Sometimes the susceptible animal may become so heavily infested with lice that death occurs. Scharff (1962) stated that the death loss resulting from very heavy infestations of the short-nosed cattle louse, Hematopinus eurysternus (Nitzsch) was probably about one percent.

The louse involved in the experiment was the short-nosed cattle louse, Hematopinus eurysternus (Nitzsch). This louse is a very important pest of beef cattle, appearing every fall and winter. According to Scharff (1962), this species is the most common louse infesting cattle in Montana during the winter months. Peterson et al. (1953) and Shemanchuk et al. (1960) demonstrated that infestations may become great enough to cause severe anemia and death to their victims. They draw so much blood that the animal's body is unable to replace it by hemopoiesis.

The purpose of this thesis is to determine if the degree of louse infestation is related to the amount of riboflavin, thiamine, niacin, and pantothenic acid in the animal's blood. Kemper (1953) stated that there may be some relationship between cattle louse infestation and the lack of some B-vitamins in the blood. Kartman

(1949) observed that rats, deficient in thiamine, riboflavin and pantothenic acid, were more susceptible to pediculosis than rats not lacking the vitamins. Matthyse (1946) suggested that the levels of vitamins A and D in the host apparently had no relationship to the infestation of cattle lice.

# LITERATURE REVIEW

Pfadt (1962) stated that the short-nosed cattle louse, H. eurysternus, most commonly infests mature animals of the beef breeds. The female lice glue their eggs on the hairs close to the skin. Craufurd-Benson (1941a) stated that in the month of January the top of the neck was the main breeding area. If the top of the neck became overcrowded, the breeding area extended down the sides of the neck. The incubation period averaged 12 days. He also reported that the time period for each of the three nymphal instars averaged four days and that the preoviposition period averaged four days. The adult males lived up to ten days while the adult females lived up to 16 days. Craufurd-Benson (1941b) found the nymphs developed on all parts of the neck while the adults and eggs were found scattered along the top of the neck. Munro and Telford (1943) found that the lice usually fed in groups in the folds of the skin and resembled patches of dirt. During their lifetime, the females laid 30 to 35 eggs depositing two or three a day.

Usually the lice do not remain on the cattle during the warm summer months while the cattle are on green grass. Kemper (1953) stated that frequently a marked reduction is noted when heavily infested animals are brought from a poor range and fed liberal rations of good hay and commercial feed concentrates. Craufurd-Benson (1941b) suggested that cattle, on poor quality rations and in a low condition of health, were more susceptible to louse infestations than cattle

on high quality rations and in a good condition of health. Kemper (1953) noted that some animals continued to be heavily infested while the others lost their lice completely. This may indicate that some nutrient in the blood of the carriers may be lacking, making the cow more desirable to the lice. Gyorgy (1938) noted pediculosis on about 20 percent of the rats kept on a riboflavin-free diet for eight weeks and reported that the pediculosis was practically eliminated by the therapeutic feeding of riboflavin. Gyorgy and Eckhardt (1940) indicated that pediculosis was common on rats with certain cutaneous lesions similar to those produced when rats received a crude concentration of vitamin $B_6$, lacking pantothenic acid.

Gojmerac et al. (1959) found that the development of high populations of cattle biting louse, Bovicola bovis (Linne), was not affected by the diameter of the cow's hair. Craufurd-Benson (1941b) stated that the thickness of the coat was more important than the thickness of the individual hairs. He believed that the thickness of the coat protected the lice from sunlight and regulated the temperature gradient between the skin of the host and the air.

Lewis and Christenson (1962) found that high populations of the B. bovis could be maintained during the summer months if the animals were not permitted to lick and rub themselves. They also found that the densities of louse populations paralleled the changes in density and length of the cow's hair coats. During the fall and winter months, the short-nosed cattle louse, H. eurysternus, may increase to large numbers which may cause a reduction in blood

cells in the infested cattle. Shemanchuk et al. (1960) found that the blood of louse-infested animals contained 2.09 to 5.0 million erythrocytes per cubic millimeter and 3.1 to 10.5 thousand leucocytes per cubic millimeter, while the blood of the louse-free animals contained 6.5 to 8.5 million erythrocytes per cubic millimeter and 7.05 to 12.1 thousand leucocytes per cubic millimeter. Schalm (1961) gave the average range of erythrocyte counts for a cow as 6.5 to 8.0 million per cubic millimeter and leucocyte counts as 7.0 to 9.5 thousand per cubic millimeter. Schalm's differential leucocyte count was: lymphocytes, 58%; neutrophils, 28%; eosinophils, 9%; monocytes, 4%; and basophils, 0.5%.

The B-vitamins are synthesized by the bacteria living in the rumen and intestinal tract of cattle. The third edition of Methods of Vitamin Assay (1966) stated that cattle apparently obtain sufficient amounts of the B-vitamins either through tissue synthesis or microbial synthesis in the digestive tract.

St. Tuzynska and Tautt (1961) studied the microbiological determination of B-vitamins in human blood. Through their experiments, they found that there were 7.6 micrograms of thiamine, 35 micrograms of riboflavin, 620 micrograms of niacin, and 21 micrograms of pantothenic acid in 100 milliliters of blood. The Handbook of Biological Data (1956) stated that there were 8.0 micrograms of thiamine, 15 to 60 micrograms of riboflavin, 15 to 45 micrograms of pantothenic acid, and 0.2 to 0.9 milligrams of niacin in 100 milliliters of whole human blood.

## HISTORY OF TEST ANIMALS

The criteria for evaluating the degree of louse infestation were based on the size of the groups of lice found on the animal. When the lice were found singly or in groups of two or three, the population was spoken of as light. The population was referred to as medium when the lice were found in groups of five to fifteen. When the groups averaged more than 15 lice, a heavy population was indicated.

This project started April 6, 1966 in Brookings County, South Dakota, when the "carrier" cow at John Collins farm was examined. The cow carried a medium to heavy population of H. eurysternus in all growth stages on her legs and front shoulders. Following this initial examination, the cow was treated with a 0.25 percent lindane dust.

The cow was again examined on April 20. At this time neck-chains, and the numbers 31 and 6 were affixed about the necks of the "carrier" cow and a herd-mate respectively. The herd-mate was chosen because she was not carrying any lice and was similar in size and appearance. Both cows were Herefords, weighing 1200 pounds and about six years old.

The cows were examined approximately every two weeks for the remainder of the spring and summer. The louse population on cow 31 became reduced to zero following the treatment with lindane. In July the number of lice increased to light infestation. The louse population remained light until cold weather when it grew to a medium infestation.

The second "carrier" cow, a six year old Hereford, was examined on April 20, 1966, at which time she was observed to be carrying a heavy infestation of H. eurysternus. Neck-chain number 2 was placed on the cow and neck-chain number 443 was placed on a louse-free herd-mate. Immediately following the first examination the owner, Robert Hornby, treated the entire herd thoroughly with a toxaphene spray consisting of five pints of 60% emulsion in 100 gallons of water.

Mr. Hornby treated his cattle for lice two and four weeks later with the same material as used on April 20. The adult and immature lice on cow number 2 were killed by the spray. The young hatching from the cow's shoulders and brisket were either killed by the chemical residue or by the next treatment. During the months of May and June no lice could be found on cow number 2. But in late July, the louse population again became evident and remained in small numbers until Mr. Hornby started spraying again in October.

The third carrier, a yearling steer belonging to South Dakota State University, was first examined May 23, 1966. The steer was heavily infested with H. eurysternus and B. bovis. The lice had made the steer anemic and unthrifty while he had been in the feed lot.

During the time between the first and second examinations, this Hereford steer and its herd-mates were sprayed with a one percent solution of lindane. The steer remained unthrifty following the spraying. After the lot of animals was moved to the college main

feed area on June 20 they were sprayed again with lindane. The examinations in July and during the remaining months of the experiment showed that the lice had been controlled by the last spraying.

The susceptible or "carrier" steer wore the ear tag 541 and the louse-free herd-mate wore the ear tag 508. These steers were purchased by the Animal Science Department of South Dakota State University in October, 1965, at Highmore, South Dakota for a feeding experiment. When the steer number 541 was placed on the feeding trial, it weighed 326 pounds. The steer was weighed again before it was brought to Brookings in May and it weighed 376 pounds. Other steers in the same feed trial gained from 75 to 100 pounds during the same period, indicating that the heavy louse population may have prevented proper weight gain.

A fourth "carrier" animal was located and first examined on June 3, 1966. Lice were found in groups about the size of a half-dollar on the cow's brisket and neck. There were nits in heavy concentrations on the shoulders of the cow. The cow was treated after examination with a locally compounded mange spray and one percent rotenone dust.

The lice that were not exposed to the treatment remained on the brisket and neck. During the examinations in June, July and August, a steady increase in louse population was noted.

Prior to the bleedings, this animal was dropped from the research project since she had become too wild to corral and handle, and the blood tests would have been inaccurate for corpuscle numbers and vitamin levels.

# METHODS AND MATERIALS

## Louse counts

Starting on March 14, 1966, animals which had histories of being susceptible to louse infestation were selected. Permission to study the animals was secured from the owners. The animals selected were three mature Hereford cows and one yearling steer. A louse-free herdmate of each susceptible animal was also selected. Three of the pairs were owned by farmers living near Brookings, South Dakota. The other pair were steers included in a nutrition study being carried on by the South Dakota State University Animal Science Department.

The cattle were examined every two or three weeks for the degree of louse infestation. The louse infestation on each animal was assessed in the following manner. The animal was restrained either by the use of a chute and headgate or by snubbing the animal to a post. The hair on the restrained animal's neck and shoulders was parted, and the number of lice per square inch was estimated. These examinations continued through the summer until the blood sampling segment of the experiment started. Once blood sampling was initiated the louse populations were estimated at every other bleeding or once a month.

Bleeding phase: The bleeding phase was started on August 23, 1966, and terminated on December 8, 1966. A volume of two hundred milliliters of blood was removed from each animal every two weeks by

means of a bleeding needle inserted into the jugular vein of the animal. The blood was collected in a wide mouth 250 milliliter Erlenmeyer flask and in a test tube containing 0.2 milliliters of one percent sodium citrate solution. The fresh blood in the test tube was used to make cell counts. The blood collection in the flask was held under refrigeration and allowed to clot. The serum was drawn off 24 hours after collection. The serum samples were then divided into eight five-milliliter samples and frozen for the later assay.

The total erythrocyte and leucocyte counts were determined, utilizing a Levy-Hauseer Corpuscle counting chamber. The differential leucocytic counts were determined, employing Wright's stain.

Vitamin Assay Phase: The amounts of riboflavin, niacin, thiamine and pantothenic acid in the blood samples were measured by microbiological assay. The samples for assay were prepared by a procedure adapted from work done by St. Tuszynska and Tautt (1961). The assays for the vitamins were performed according to the procedure outlined in the Difco Manual (1964).

Method of Riboflavin Assay

Sample Preparation: A frozen five milliliter sample from each of the bleedings was thawed by placing the tubes in a basin of warm water. The samples were hydrolyzed by the addition of 20 milliliters of 0.1 normal hydrochloric acid to each sample. The samples were autoclaved for 15 minutes under one atmosphere of pressure. The pH of each sample was adjusted to 4.5. One milliliter of a ten percent solution of papain and malt amylase was added to each sample.

The samples were digested by the enzymes for 18 hours at 37 degrees centigrade. The enzymes were then inactivated by heating the samples to 100 degrees centigrade. The samples were increased to a volume of 80 milliliters and filtered. The dilution of the samples to the above volume adjusted the riboflavin concentration to an approximate concentration of 0.1 micrograms per milliliter.

When this preparation was filtered for the final time, not all of the protein fragments and amino acids were removed. A precipitate was found in several of the samples following the addition of the riboflavin-free medium and the autoclaving of the samples. Three assay tubes of each sample were prepared in order to allow for the precipitation in the tubes. Two were inoculated and the other was not. The blank reading was subtracted from the average reading of the two inoculated tubes.

The riboflavin preparation was performed twice, 38 samples each time. This procedure gave a duplicate reading for each bleeding.

Microbiological Assay: Stock cultures of Lactobacillus casei were prepared by stab inoculation into ten milliliter tubes of Bacto-Micro Assay Culture Agar. The stock cultures were maintained by 24 hour transplants of the culture. The inoculum for assay was prepared by subculturing from the stock culture into ten milliliters of Bacto-Micro Inoculum Broth. Following incubation for 24 hours at 35 to 37 degrees centigrade, the broth was centrifuged, under aseptic conditions, and the supernatant liquid decanted. The cells were

resuspended in ten milliliters of sterile isotonic sodium chloride. The cell suspension was then diluted one to twenty with sterile isotonic sodium chloride. One drop of this latter suspension was then used to inoculate each of the assay tubes.

Turbidometric readings were made after 24 hours of incubation at 35 to 37 degrees Centigrade.

The concentrations of riboflavin required for the preparation of the standard curve, Figure 1, was prepared by dissolving 0.1 gram of riboflavin in 1000 milliliters of distilled water by heating, giving a stock solution of 100 micrograms per milliliter. The stock solution was diluted by adding one milliliter to 999 milliliters of distilled water, and 0.0, 0.25, 0.5, 0.75, 1.0, 1.5, 2.0 and 3.0 milliliters of the diluted stock solution were used per tube. The riboflavin was then at levels of 0.0, 0.025, 0.05, 0.075, 0.1, 0.15, 0.2 and 0.3 micrograms of riboflavin per tube.

A riboflavin-free basal medium was added to each tube of sample. The basal medium was formed by the suspension of 33.6 grams of Bacto-Riboflavin Assay Medium in 700 milliliters of distilled water and heating to boiling. For the assay, each tube contained five milliliters of rehydrated medium, increasing amounts of standard or unknown, and sufficient distilled water to give a total volume of ten milliliters per tube. The tubes were then autoclaved for ten minutes at 15 pounds pressure, cooled and inoculated as previously described.

Method of Niacin Assay

Sample Preparation: A five milliliter sample from each bleeding date was thawed in warm water. Thirty-five milliliters of distilled water was added to the sample. The sample was then deproteinized by the following series of steps. Ten milliliters of five percent aluminum sulfate solution was added and the sample was held for 45 minutes in the dark. One gram of finely ground calcium carbonate was added and the sample was permitted to stand for 15 minutes. The sample was refluxed for four minutes within a 250 milliliter Erlenmeyer flask topped with a piece of aluminum foil and filtered while hot. The filtrate was concentrated to ten milliliters in a forced air drying over set at 70 degrees centigrade. The sample was refiltered and the pH was adjusted to 5.2. The volume was increased to 125 milliliters with distilled water to produce the final niacin concentration of about one microgram per milliliter.

Microbiological Assay: Stock cultures of Lactobacillus plantarum were prepared by stab inoculation of Bacto-Micro Assay Culture Agar. The culture was transplanted at 24 hour intervals in order to maintain a good, growing stock culture. The inoculum for assay was prepared by subculturing from a stock culture into a tube containing ten milliliters of Bacto-Micro Inoculum Broth. After 24 hours of incubation at 35 to 37 degrees centigrade, the cells were centrifuged under aseptic conditions and the supernatant liquid decanted. The cells were resuspended in ten milliliters of sterile

isotonic sodium chloride. The cell suspension was then diluted one to one hundred with sterile isotonic sodium chloride. One drop of this suspension was then used to inoculate each of the assay tubes.

A standard curve was set up for each assay since conditions of autoclaving and various other factors influenced the standard curve readings from test to test. The concentrations of niacin required for the preparation of the standard curve (Figure 2) were prepared by dissolving 0.1 gram of niacin in one liter of distilled water, giving a stock solution of 100 micrograms per milliliter. The stock solution was diluted by adding one milliliter to 999 milliliters of distilled water. The standard curve was obtained by using 0.0, 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 milliliters per tube, giving levels of 0.0, 0.05, 0.1, 0.2, 0.3, 0.4 and 0.5 micrograms of niacin per assay tube.

For the assay, each tube contained five milliliters of rehydrated medium increasing amounts of the standard or the unknown and sufficient distilled water to give a total volume of ten milliliters per tube. The basal medium was rehydrated by suspending 52.5 grams of Bacto-Niacin Assay Medium in 700 milliliters of distilled water and heating the suspension to the boiling point.

The tubes were then autoclaved for ten minutes at 15 pounds pressure. Next the tubes were inoculated and then incubated for 18 hours at 35 to 37 degrees centigrade. The contents of the tubes were then read for turbidity at a wave length of 525 millimicrons with a Bausch and Lomb spectrophotometer.

Method of Pantothenic Acid Assay

Sample Preparation: The procedure, outlined by St. Tuszynska and Tautt (1961), used materials that were not accessible for the experiment. It called for liver autolyzate as the enzyme in the digestion of the blood proteins. This material does contain bound calcium pantothenate and would have influenced the assay to some extent. The above indicated procedure would probably have been satisfactory if there had been sufficient time to determine the amount of calcium pantothenate in the liver autolyzate. The procedure used in the preparation of niacin assay samples was adapted for use in this sample preparation.

The five milliter samples were thawed. Thirty-five milliters of distilled water were added to each sample. Then ten milliters of five percent aluminum sulfate were added. After 45 minutes, one gram of ground calcium carbonate was added and the sample was permitted to stand for 15 minutes. The sample was refluxed on an electric hot plate for four minutes. The sample was filtered while it was still hot. The sample was then concentrated to ten millliliters in the forced air drying oven and refiltered. The volume of the filtrate was increased to 20 milliliters to obtain a calcium pantothenate concentration of 0.10 micrograms per milliliter.

Microbiological Assay: Stock cultures of the test organism, Lactobecillus plantarum, were prepared by stab inoculation of five milliliter tubes of Bacto-Micro Assay Culture Agar. The stock culture was maintained in a growing state by making transplants at 24 hour

intervals. The inoculum for assay was prepared by subculturing from a growing stock culture of L. plantarum into a tube containing ten milliliters of Bacto-Micro Inoculum Broth. Following incubation for 24 hours at 35 to 37 degrees Centigrade, the cells were centrifuged, under aseptic conditions, and the supernatant liquid decanted. The cells were resuspended in ten milliliters of sterile isotonic sodium chloride. The cell suspension is then diluted one to one hundred milliliters with sterile isotonic sodium chloride. A single drop of the suspension was used to inoculate each of the assay tubes.

The concentrations of calcium pantothenate, necessary for the preparation of the standard curve, were prepared by dissolving 0.1 gram of calcium pantothenate in 1000 milliliters of distilled water, giving a stock solution of 100 micrograms per milliliter. The stock solution was diluted by adding one milliliter to 99 milliliters of distilled water. This solution was further diluted by adding four milliliters to 96 milliliters of distilled water to receive the final solution. The standard curve was obtained by using 0.0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 and 5.0 milliliters per tube. The calcium pantothenate was then at levels of 0.0, 0.02, 0.04, 0.06, 0.08, 0.1, 0.12 and 0.2 micrograms per assay tube. The standard curve is necessary since conditions of autoclaving, temperature of incubation, and other factors which influence the standard curve readings can not be duplicated from test to test.

For the assay, each tube had to contain five milliliters of rehydrated medium, the unknown or increasing amounts of the standard, and sufficient distilled water to give a total volume of ten milliliters per tube. The rehydrated medium was produced by suspending 36.5 grams of Bacto-Pantothenate Assay Medium in 500 milliliters of distilled water and heating to a boil for three minutes.

The tubes were autoclaved for ten minutes at 15 pounds pressure. When the tubes had cooled, they were inoculated and incubated for 18 hours at 37 degrees centigrade. The optical density of each tube was determined at 525 millimicrons in a Bausch and Lomb spectrophotometer. Values for optical density were read from the standard curve, Figure 3, as micrograms of pantothenic acid.

Method of Thiamine Assay

Sample Preparation: The five milliliter serum samples were thawed by placing the rack of sample tubes in a pan of warm water. Twenty milliliters of 0.1 normal hydrochloric acid were added to each sample. The mixture was hydrolyzed for 30 minutes in a steam cabinet. The pH of each sample was adjusted to 4.5 with a one normal solution of sodium hydroxide. A pH meter with a single probe was used since it enabled the reading of the samples in Erlenmeyer flasks. One milliliter of a ten percent enzyme solution containing papain and melt amylase was added to each sample. The samples were digested for 18 hours at 37 degrees centigrade. The samples were heated to 100 degrees centigrade to inactivate the enzymes. The mixture was then

filtered and the filtrate was adjusted to a pH of 6.2. The sample was diluted to a volume of 100 milliliters to reach a concentration of 0.01 micrograms of thiamine per milliliter.

Microbiological Assay Procedure: A standard curve of thiamine was produced, Figure 4. The concentrations of thiamine required for the preparation of the standard curve was prepared by dissolving 0.1 gram of thiamine hydrochloride in one liter of distilled water, giving a stock solution of 100 micrograms per milliliter. Dilution of the stock solution was accomplished by adding one milliliter to 99 milliliters of distilled water to give the final solution. The standard curve was prepared by using 0.0, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0 and 5.0 milliliters of this final solution per tube. Five milliliters of rehydrated medium had also been added to each of the tubes for the standard curve and to each tube containing material under assay. The rehydrated medium was prepared by suspending 42.5 grams of Bacto-Thiamine Assay Medium in 500 milliliters of distilled water and heating to boiling for three minutes. Sufficient water was added to the standard curve tubes to bring the volume in each tube up to ten milliliters. The tubes for the standard curve then contained 0.00, 0.005, 0.01, 0.015, 0.02, 0.03, 0.04, and 0.05 micrograms of thiamine hydrochloride per tube, Figure 4.

Stock cultures of the test organism, Lactobacillus fermentum, were prepared by stab inoculation of Bacto-Micro Assay Culture Agar. The culture was maintained by transplants every 24 hours.

The inoculum for the assay was prepared by subculturing from a stock to ten milliliters of Bacto-Micro Inoculum Broth. After 18 hours of incubation at 37 degrees centigrade, the cells were centrifuged, under aseptic conditions, and the supernatant liquid was decanted. The cells were resuspended in ten milliliters of sterile isotonic sodium chloride. One half milliliter of this suspension was then added to 100 milliliters of sterile isotonic saline. One drop of this suspension was then used to inoculate each of the assay tubes.

Following inoculation, the tubes were incubated at 37 degrees centigrade for 18 hours, and then refrigerated for 15 minutes in order to stop growth. The tubes were then read for optical density in the spectrophotometer at 525 millimicrons. The micrograms of thiamine were read from the standard curve.

## RESULTS AND DISCUSSION

The louse counts during this experiment indicated that the short-nosed cattle louse, Hematopinus eurysternus (Nitzsch), prefers the front shoulders and the top of the neck as an oviposition area. The louse populations of adults and nymphs occurred in areas other than those reported by Craufurd-Benson (1941b). The adults were observed on the lower neck, and the nymphs were found on the shoulders and lower neck. They also preferred to feed in groups, presumably near a major surface blood vessel. The time of year for these observations may explain why the feeding sites differed from those observed by Craufurd-Benson (1941b). Craufurd-Benson (1941b) made his observations during the months of December through February. The observations in this experiment were made during the months from April to September.

The louse counts also indicated that H. eurysternus can be a very persistant pest of susceptible animals during the summer months. All of the cattle in this experiment were treated very thoroughly with chemicals for louse control. However, when the residual effect of the treatments had dissipated, light populations of lice developed on each of the "carrier" animals.

Blood cell counts showed higher leucocyte and lower erythrocyte levels in the "carrier" animals when compared to the louse-free animals. The average number of leucocytes was 5.7 thousand per cubic millimeter in the "carriers" and 5.6 thousand per cubic millimeter in the louse-free animals. The average number of erythrocytes was 5.5

million per cubic millimeter in the carrier animals and 5.8 million per cubic millimeter in the louse-free animals. This ratio is less than that observed by Shemanchuk et al. (1960). Their observations, however, were made in the comparison of heavily infested animals with louse-free animals. The results of the blood counts are shown in the first three tables.

The parasitized animals could be recognized by their differential leucocyte counts. The percentage of eosinophils was high in the infested animals, suggesting the presence of foreign toxins in the blood.

The results of the experimental phase utilizing standard microbiological assay procedures to determine the levels of thiamine, riboflavin, pantothenic acid, and niacin indicated that there was very little difference in these blood vitamin levels between the "carriers" and the louse-free animals. The 12 samples assayed for each vitamin level in the blood of each animal showed variation from sampling date to sampling date. These variations could have been due to the fluctuation of the vitamin level in the blood or variations in the laboratory technique. When the turbidity readings of blood samples for the individual vitamins in each animal were averaged and compared to the respective standard curves (Figures 1, 2, 3, and 4), the average vitamin levels (Table 4) showed only individual differences among the animals.

# CONCLUSIONS

The data determined in this experiment indicated that during the summer months "carrier" animals, when their louse infestations were controlled, could still be identified by lower erythrocyte levels than those found in the louse-free animals. However, the average vitamin levels did not show differences in thiamine, pantothenic acid, niacin, and riboflavin which could be correlated with "carrier" or louse-free conditions.

Table 1. Erythrocyte and leucocyte data on blood samples taken from a "carrier" steer and a louse-free steer in the South Dakota State University herd in 1966.

| | Sampling Dates | | | | | |
|---|---|---|---|---|---|---|
| | 8-30 | 9-15 | 10-12 | 10-27 | 11-10 | Average |
| Animal #541 ("carrier") | | | | | | |
| Erythrocytes (millions/c.mm.) | 6.6 | 6.4 | 5.5 | 6.0 | 5.7 | 6.0 |
| Leucocytes (thousands/c.mm.) | 7.7 | 9.0 | 6.4 | 5.4 | 5.5 | 6.8 |
| Lymphocytes (%) | 68 | 93 | 76 | 69 | 88 | 78.8 |
| Monocytes (%) | 3 | 2 | 3 | 2 | 0 | 2.0 |
| Neutrophils (%) | 28 | 5 | 20 | 28 | 10 | 18.2 |
| Eosinophils (%) | 1 | 0 | 1 | 1 | 1 | 0.8 |
| Basophils (%) | 0 | 0 | 0 | 0 | 1 | 0.2 |
| Animal #508 (louse-free) | | | | | | |
| Erythrocytes (millions/c.mm.) | 8.3 | 6.4 | 5.9 | 5.3 | 6.4 | 6.5 |
| Leucocytes (thousands/c.mm.) | 7.8 | 8.4 | 8.2 | 4.6 | 7.9 | 7.4 |
| Lymphocytes (%) | 77 | 75 | 53 | 87 | 60 | 70.4 |
| Monocytes (%) | 4 | 2 | 2 | 2 | 0 | 2.0 |
| Neutrophils (%) | 17 | 23 | 40 | 9 | 39 | 25.6 |
| Eosinophils (%) | 2 | 0 | 5 | 2 | 1 | 2.0 |
| Basophils (%) | 0 | 0 | 0 | 0 | 0 | 0.0 |

Table 2. Erythrocyte and leucocyte data on blood samples from a "carrier" cow and a louse-free cow in the Robert Hornby farm herd in 1966.

| | Sampling Dates 8-23 | 9-21 | 10-7 | 10-22 | 11-7 | Average |
|---|---|---|---|---|---|---|
| **Animal #2 ("carrier")** | | | | | | |
| Erythrocytes (millions/c.mm.) | 5.6 | 5.1 | 5.6 | 5.6 | 4.9 | 5.4 |
| Leucocytes (thousands/c.mm.) | 6.0 | 5.2 | 6.8 | 5.0 | 5.1 | 5.6 |
| Lymphocytes (%) | 57 | 75 | 49 | 55 | 86 | 64.4 |
| Monocytes (%) | 14 | 3 | 2 | 1 | 3 | 4.6 |
| Neutrophils (%) | 22 | 21 | 24 | 23 | 10 | 20.0 |
| Eosinophils (%) | 7 | 1 | 23 | 20 | 1 | 10.4 |
| Basophils (%) | 0 | 0 | 2 | 1 | 0 | 0.6 |
| **Animal #443 (louse-free)** | | | | | | |
| Erythrocytes (millions/c.mm.) | 7.1 | 7.2 | 5.7 | 5.9 | 5.0 | 6.2 |
| Leucocytes (thousands/c.mm.) | 8.8 | 6.4 | 4.8 | 5.0 | 4.6 | 5.9 |
| Lymphocytes (%) | 60 | 78 | 82 | 89 | 84 | 78.6 |
| Monocytes (%) | 6 | 3 | 3 | 3 | 1 | 3.2 |
| Neutrophils (%) | 20 | 19 | 11 | 2 | 14 | 13.2 |
| Eosinophils (%) | 14 | 0 | 4 | 4 | 1 | 4.6 |
| Basophils (%) | 0 | 0 | 0 | 2 | 0 | 0.4 |

Table 3. Erythrocyte and leucocyte data on blood samples taken from a "carrier" cow and a louse-free cow in the John Collins farm herd in 1966.

| | Sampling Dates | | | | | |
|---|---|---|---|---|---|---|
| | 8-23 | 9-23 | 10-15 | 10-27 | 11-14 | Average |
| Animal #31 ("carrier") | | | | | | |
| Erythrocytes (millions/c.mm.) | 6.1 | 4.3 | 5.9 | 5.3 | 5.6 | 5.4 |
| Leucocytes (thousands/c.mm.) | 5.4 | 5.2 | 4.8 | 5.4 | 5.5 | 5.3 |
| Lymphocytes (%) | 77 | 92 | 43 | 60 | 40 | 62.4 |
| Monocytes (%) | 4 | 0 | 0 | 3 | 4 | 2.2 |
| Neutrophils (%) | 13 | 8 | 42 | 21 | 38 | 24.4 |
| Eosinophils (%) | 4 | 0 | 15 | 15 | 18 | 10.4 |
| Basophils (%) | 2 | 0 | 0 | 1 | 0 | 0.6 |
| Animal #6 (louse-free) | | | | | | |
| Erthrocytes (millions/c.mm.) | 6.1 | 7.5 | 5.5 | 5.6 | 5.0 | 5.9 |
| Leucocytes (thousands/c.mm.) | 7.0 | 4.4 | 4.6 | 3.8 | 5.1 | 5.0 |
| Lymphocytes (%) | 68 | 82 | 71 | 67 | 55 | 68.6 |
| Monocytes (%) | 11 | 2 | 0 | 5 | 0 | 3.6 |
| Neutrophils (%) | 21 | 15 | 12 | 16 | 33 | 19.4 |
| Eosinophils (%) | 10 | 1 | 17 | 12 | 12 | 10.4 |
| Basophils (%) | 0 | 0 | 0 | 0 | 0 | 0.0 |

Table 4. The average blood levels for the microbiological assays for riboflavin, niacin, pantothenic acid, and thiamine.

| Animal Number | Average[1] ug/ml of blood serum | | | |
|---|---|---|---|---|
| | Riboflavin | Niacin | Pantothenic Acid | Thiamine |
| 2 ("carrier") | .077 | 2.15 | .099 | .36 |
| 443 (louse-free) | .064 | 2.5 | .109 | .33 |
| 31 ("carrier") | .038 | 1.75 | .061 | .22 |
| 6 (louse-free) | .064 | 2.0 | .086 | .33 |
| 541 ("carrier") | .051 | 3.4 | .246 | .22 |
| 508 (louse-free) | .038 | 2.75 | .166 | .22 |

[1]The average of six samples assayed in duplicate.

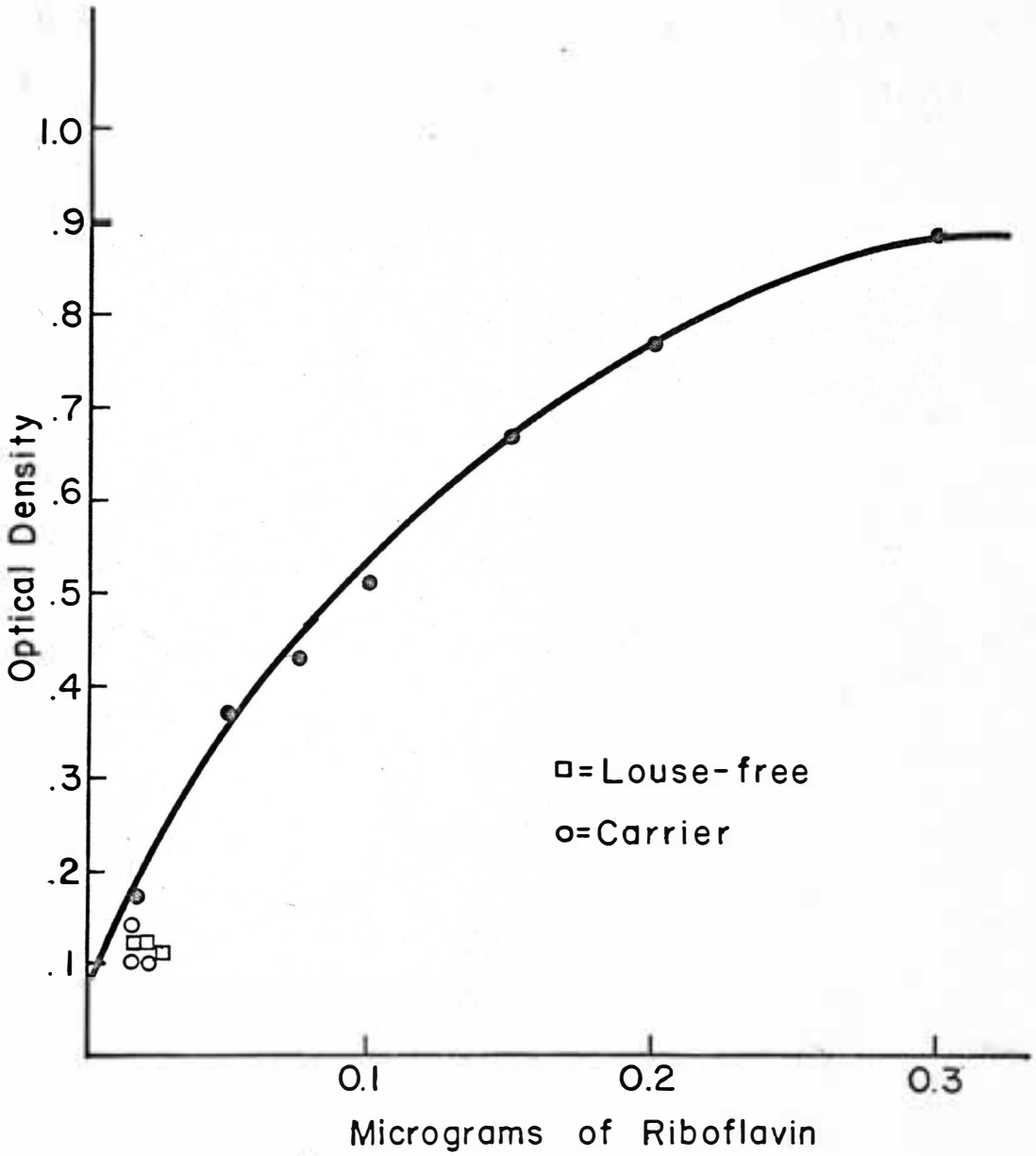


FIG. 1 Comparison of blood riboflavin levels in "carrier" and louse-free cattle in relation to the growth response of Lactobacillus casei to a standard riboflavin dilution.

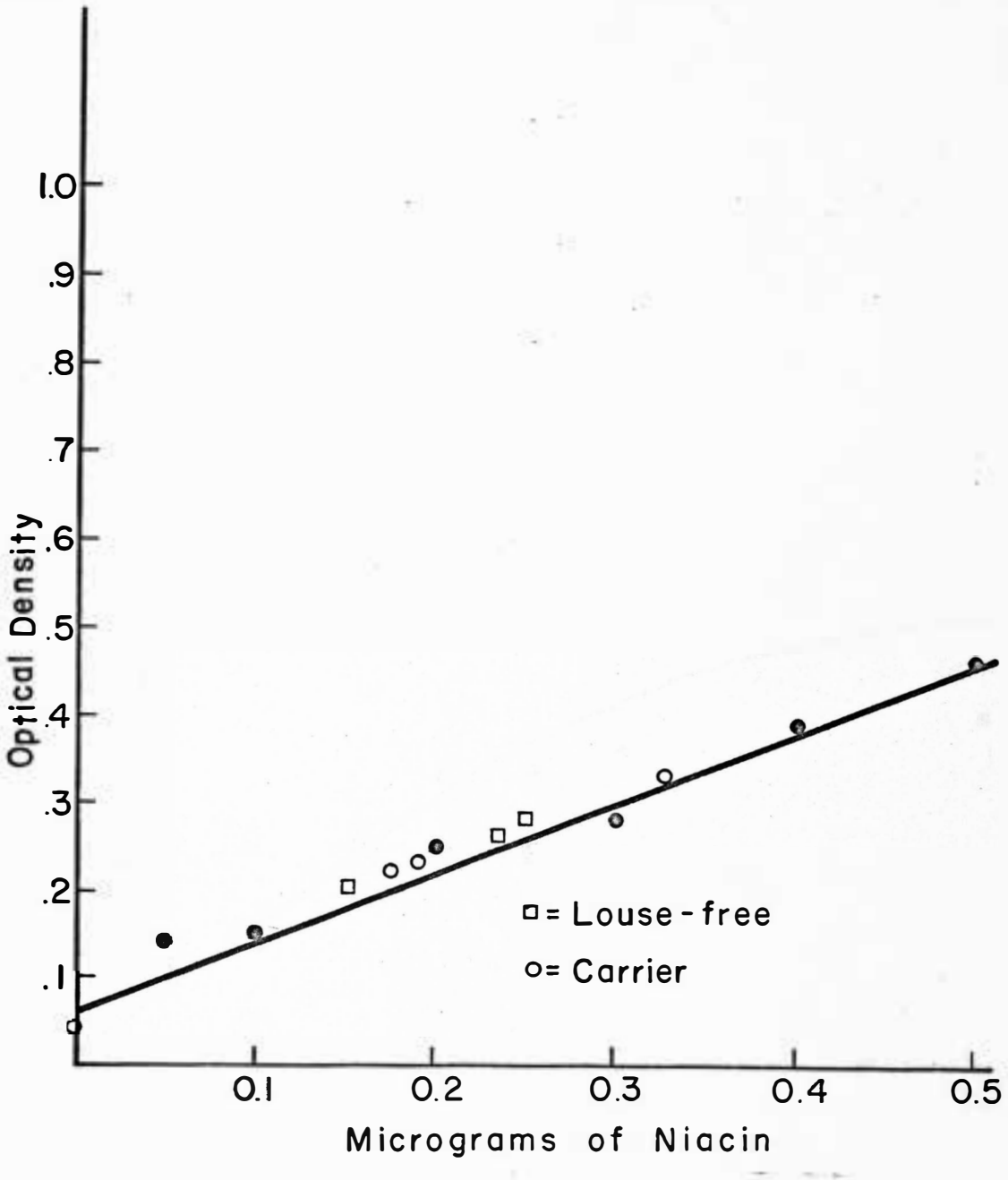


FIG. 2 Comparison of blood niacin levels in "carrier" and louse-free cattle in relation to the growth response of Lactobacillus plantarum to a standard niacin dilution.

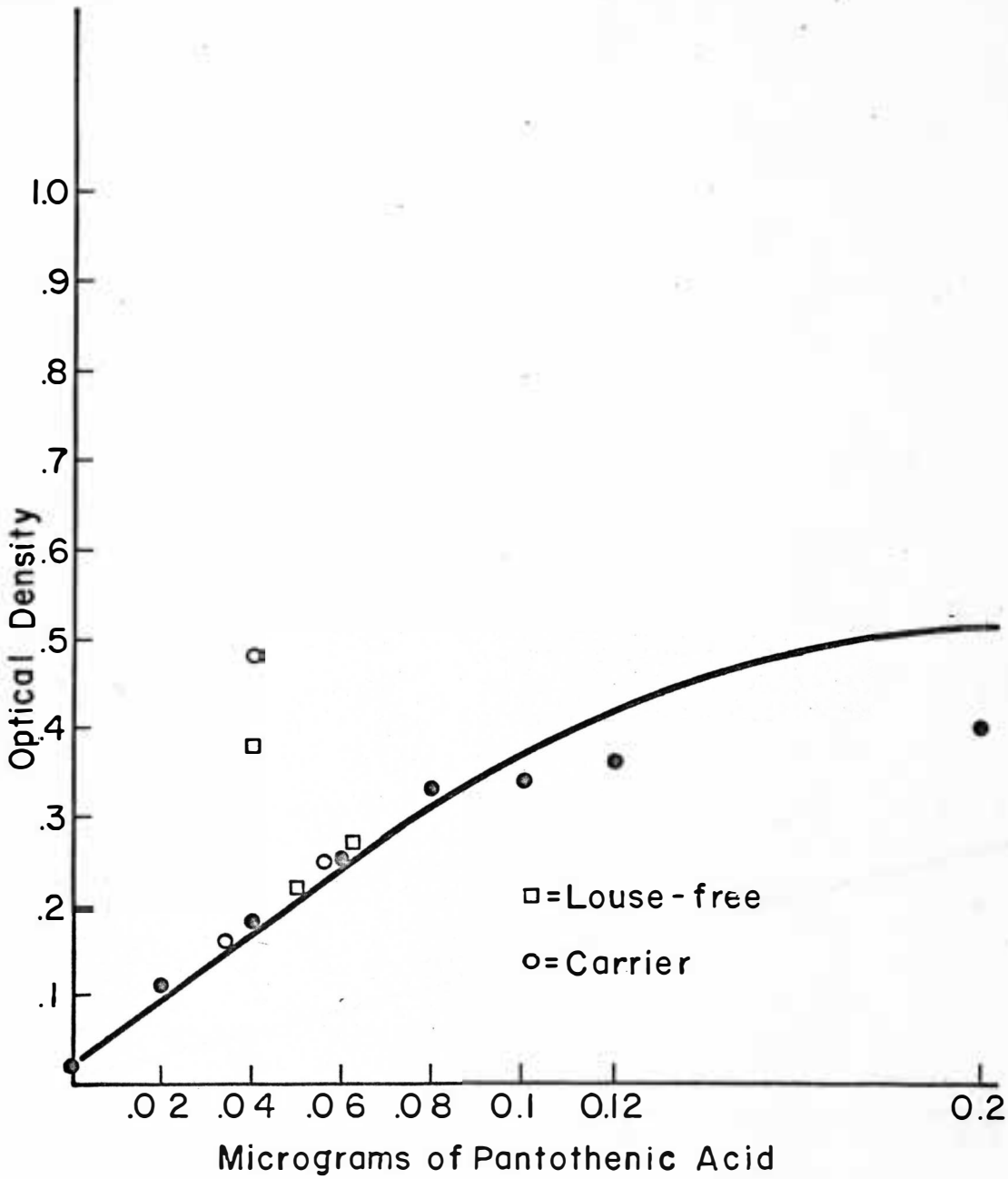


FIG. 3 Comparison of blood pantothenic acid levels in "carrier" and louse-free cattle in relation to the growth response of Lactobacillus plantarum to a standard pantothenic acid dilution.

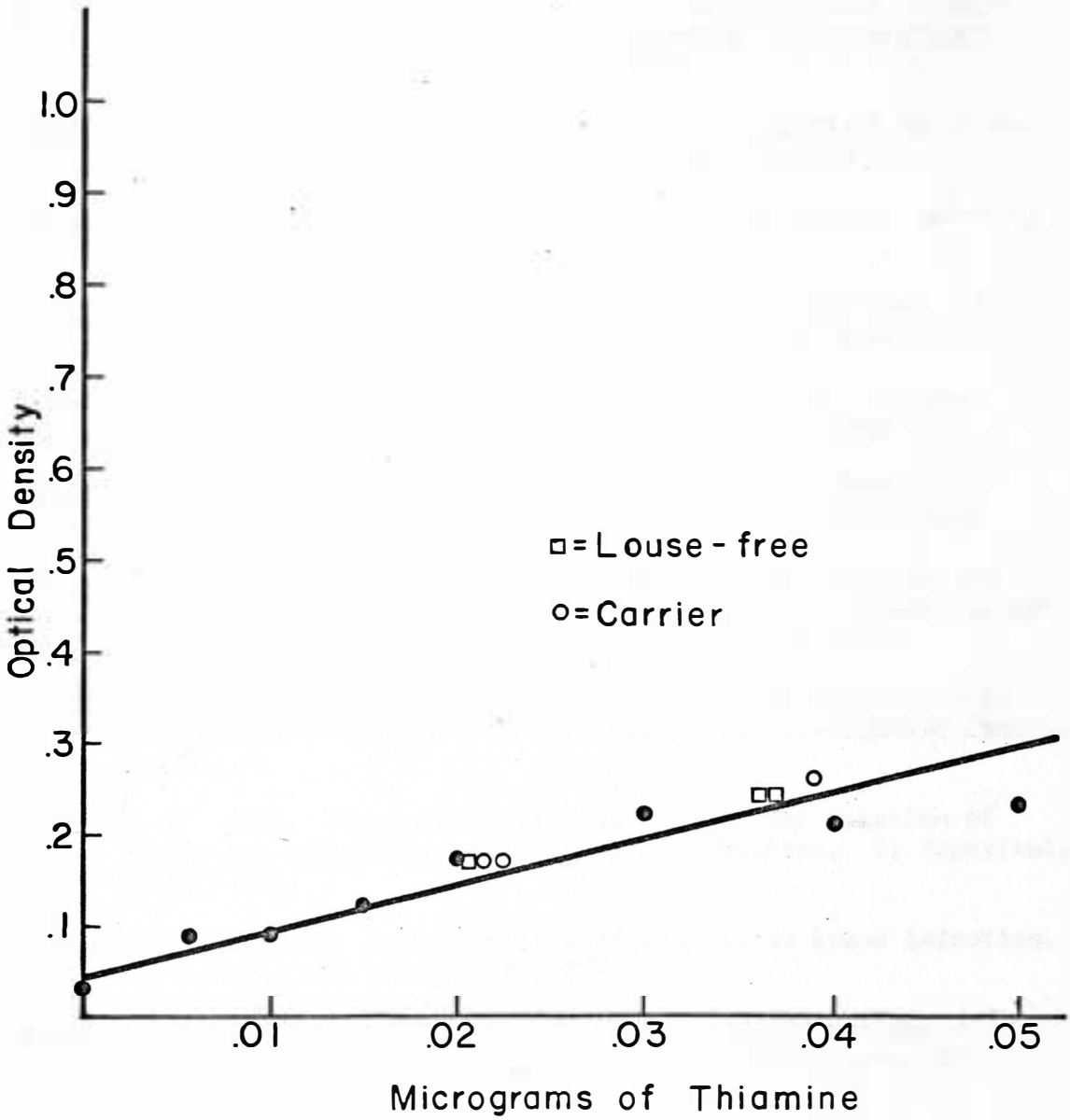


FIG. 4 Comparison of blood thiamine levels in "carrier" and louse-free cattle in relation to the growth response of Lactobacillus fermentii to a standard thiamine dilution.

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