ABSTRACT OF THESIS

EFFECTS OF NITRATES AND NITRITES ON THE DEPLETION OF LIVER VITAMIN A STORES AND CAROTENE UTILIZATION IN GROWING-FINISHING PIGS

Three trials were conducted using one hundred thirty Hampshire, Yorkshire and Hampshire x Yorkshire pigs to study the effects of nitrates (KNO_3) and nitrites (KNO_2) in rations on the depletion of liver vitamin A stores and the utilization of carotene in growing-finishing pigs. In Trial I, 50 Yorkshire x Hampshire pigs were allotted to five ration treatments to study the effects of nitrates and nitrites on the depletion of liver vitamin A stores. Prior to the start of the trial the pigs were fed a balanced ration receiving supplemental vitamin A until they reached an average weight of 100 pounds. In Trial II and III, 80 Hampshire and Yorkshire pigs were assigned to five ration treatments to study the effects of nitrates and nitrites, respectively, on the utilization of carotene in growing-finishing pigs. The sources of vitamin A activity for both trials were beta-carotene (3,300 I.U./kg.) for Treatments 1 through 4 and vitamin A palmitate (1,100 I.U./kg.) for Treatment 5. Criteria used to evaluate the experiments were liver vitamin A values (mcg./gm.), plasma vitamin A values (mcg./100 ml.), methemoglobin (gm./100 ml.), hemoglobin (gm./100 ml.), hematocrit (per cent) and the performance (lb.).

The feeding of 3% nitrate or 0.3% nitrite significantly ($P < .01$) depressed gains with or without vitamin A and carotene supplementation. Neither nitrate nor nitrite showed a significant effect on liver vitamin A stores, plasma vitamin A and methemoglobin, regardless of vitamin A source fed. The addition of 3% nitrate to the diet of pigs receiving supplemental carotene or vitamin A did not effect liver vitamin A stores, plasma vitamin A and methemoglobin levels. Plasma vitamin A levels were significantly reduced ($P < .05$) by the feeding of 0.3% nitrite receiving either supplemental carotene or vitamin A. Ration containing 0.3% nitrite with vitamin A or carotene supplementation significantly increased ($P < .01$) methemoglobin values. Nitrate (3% NO_3) or nitrite (0.3% NO_2) fed to pigs receiving supplemental vitamin A tended to show a faster depletion rate of liver vitamin A stores and a reduction of plasma vitamin A when compared to nitrate or nitrite-fed pigs receiving supplemental carotene. Hemoglobin and hematocrit remained relatively consistent for the entire trials.

(Rudy I. Hutagalung)

October 21, 1966

(Date)

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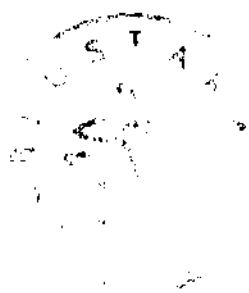
By

Rudy I. Hutagalung



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THESIS

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EFFECTS OF NITRATES AND NITRITES ON THE DEPLETION OF LIVER VITAMIN A STORES AND CAROTENE UTILIZATION IN GROWING-FINISHING PIGS

THESIS

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at the University of Kentucky

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CHAPTER I

INTRODUCTION

The important role of vitamin A and its precursors has been known for a long time in practical and also under experimental conditions. Animals inadequately supplied with vitamin A, either preformed or as its provitamins, will fail to thrive and so cause wastage of feed, labor and land which are devoted to their production. There may also be deterioration in the quality of animal product used as human food. In this respect an adequate supply of vitamin A has repeatedly been shown to be of greatest importance. In the swine industry, the latter problem seems more urgent, as pigs normally receive feed which is stored for various lengths of time may be low in vitamin A activity.

The intensive use of nitrogen fertilizers results in high nitrate levels in feeds and forages which are known to interfere with vitamin A metabolism. Drought, heat, excessive crowding, shading, and water-logged soils are believed to contribute to it.

The present day ration for swine is mostly grain. However, forages consumed by swine are often harvested from fields which have been highly fertilized with nitrogen. This can be a factor responsible for precipitating vitamin A deficiencies in swine receiving levels of carotene thought to be adequate.

Experimental evidence has been presented by O'Dell et al. (1960) showing that the feeding of dietary nitrate or nitrite caused

an increased depletion rate of vitamin A stores in rats. An adverse effect upon the vitamin A status in sheep fed dietary nitrate or nitrite has been noted (Goodrich et al., 1962, 1964; Hatfield et al., 1963, Holst et al., 1961; Smith et al., 1961). Only limited work of this nature has been reported in swine.

The experiments reported herein were conducted to determine the effects of rations containing added potassium nitrate or nitrite on: 1. the depletion of vitamin A in the liver, 2. utilization of carotene and 3. performance of young growing finishing pigs.

CHAPTER II

REVIEW OF LITERATURE

History of Vitamin A and Carotene

An ancient Egyptian medical treatise, Eber's Papyrus, of about 1500 B.C. recognized the existence of vitamin A (Aykroyd, cited by Moore, 1957), by the recommendation of roast ox-liver, or the liver of a black cock, as a factor capable of correcting an inability to see properly at night. Hippocrates, the famous Greek philosopher also prescribed raw ox-liver after dipping in honey as a cure for this ailment. Present knowledge speculates that these were early attempts to cure night blindness with the vitamin A stored in the liver.

Working at Yale University, Osborne and Mendel (1913) established the necessity of a substance in butter to promote rapid growth. At about the same time McCollum and Davis (1913) reported the prompt resumption of growth in rats, after a period of retarded growth, from feeding the ether extract of butter or eggs. They employed rations compounded of pure casein, carbohydrates, lard and a salt mixture which sustained growth for 70 to 120 days. No result in growth was obtained from feeding lard or olive oil. Thus, it was not merely the absence of fats from the diet which causes the suspension of growth. Hart and McCollum (1914) observed when swine was restricted to corn meal or wheat, little or no growth can be

secured. The addition of salts, butterfat and casein to the ration greatly improved the growth rate. McCollum and Davis (1915) reported the existence of two factors of unknown accessory substances necessary for normal nutrition. One of these was soluble in water and the other was soluble in fats. From this work came the names of fat-soluble A and water-soluble B, known as vitamins today.

Physical and Chemical Properties of Carotene and Vitamin A

Carotenoids had intensified interest of organic chemists long before the discovery of vitamins. In 1931 Karrer commenced his classical work on the distribution and chemical nature of the carotenoid pigments. He postulated the correct structural formula $C_{40}H_{56}$, that is highly unsaturated carbon, consisting of a long central unsaturated chain. When the purity of beta-carotene was established, Karrer prepared sufficient quantities to be used as an international standard.

Carotene is readily soluble in carbon disulphide, chloroform and benzene, but other organic solvents can dissolve only very limited amounts. Beta-carotene remains stable for years when it is stored *in vacuo* or in atmosphere of inert gas (Moore, 1957).

The chemical methods similar to those used for carotene eventually led Karrer to a formula of $C_{20}H_{30}O$ for vitamin A. The structure, represents one half carotene molecule combined with one molecule of water to form a terminal hydroxyl group.

Holmes and Corbett first succeeded in crystallizing the vitamin by itself. The first claim to have achieved the synthesis was made

in 1937 by Kuhn and Morris and it was not until ten years later that Arens and Van Dorp succeeded in producing the vitamin in substantial quantities in pure form.

The vitamin is soluble in fats, and in all usual organic solvents. It is insoluble in water, but may be dispersed in the aqueous phase either by emulsification or by attachment to protein.

The synthesis of carotene also took many years to accomplish, but eventually in 1950, Karrer was successful in the synthesis of beta-carotene and several other pigments.

The vitamin A activity of food is expressed in International Units (I.U.). One I.U. is defined as the activity of 0.3 mcg. of crystalline vitamin A alcohol (0.344 mcg. of vitamin A acetate). Beta-carotene is the standard for provitamin A, 0.6 mcg. being equivalent in activity to 0.3 mcg. of vitamin A (Maynard and Loosli, 1962).

The Conversion of Carotene to Vitamin A

The conversion of beta-carotene to vitamin A, which requires the relation of the molecule to half its original size and the addition of water, may seem a very simple process but even for 25 years after the discovery of the conversion, however, the mechanism underlying the change has not been worked out in full detail. The pigment is first oxidized to two molecules, or possibly only one molecule, of vitamin A aldehyde. The aldehyde is then promptly reduced to vitamin A alcohol (Moore, 1957).

It was thought at first that the conversion was effected in the liver and this view went unchallenged for many years. The application of modern chromatography methods demonstrated clearly that the conversion takes place in the intestinal walls. Although the small intestine is the most important organ involved, other tissues are also capable of carrying out this process (Oswald, 1966). In a recent paper, Zachman and Olson (1963) have shown that the isolated perfused rat liver can convert C^{14} - labelled beta-carotene to C^{14} - labelled vitamin A and that the liver can store this C^{14} - labelled beta-carotene.

Moore (1929, 1930) demonstrated that when beta-carotene was fed to rats depleted of vitamin A, not only did they resume growth but their livers accumulated a colorless material when treated with the antimony trichloride reagent. Modern knowledge indicates that plant tissues do not contain even traces of vitamin A. By the same procedure as had been used by Moore (1929, 1930) for rats, Capper et al. (1931) demonstrated the conversion of carotene to vitamin A in chickens. Collison et al. (1929) fractionated and crystallized pigments from green cabbage, spinach and carrots and found the vitamin A activity to be associated with the carotenoids.

Olcott and McCann (1931) observed the appearance of an absorption band at 325 mu when a colloidal solution of carotene was incubated with ground liver tissues taken from rats deficient in vitamin A. They assumed the presence of enzyme "carotenase," which could convert carotene into vitamin.

Woolf and Moore (1932) discounted the work of Olcott and McCann claiming their proof of carotene was not conclusive.

Drummond and McWalter (1935) were unsuccessful with liver incubations to demonstrate this conversion even by allowing rabbits to absorb carotene into their livers just before they were killed.

Sexton et al. (1946) found that parenteral injection of carotene failed to increase liver storage of vitamin A in rats. They suggested that the conversion of carotene to vitamin A may be an extra-hepatic function in the rat. Later Goodwin and Gregory (1948) concluded that the carotene was converted to vitamin A in the small intestine.

Bieri and Pollard (1954) concluded that carotene can be converted to the vitamin in tissues other than the small intestine and suggested that the liver is the main site of conversion when the provitamin is given by intravenous injection.

In rats and pigs oral administration of carotene caused the prompt appearance of vitamin A in the small intestine and blood plasma (Thompson et al., 1947). The increased blood plasma vitamin A was in esterified form.

Braude et al. (1941) reported that pigs made less efficient use of beta-carotene than do rats and efficiency of conversion of beta-carotene into vitamin A in pigs was not greater than 30 to 40 per cent.

Myers et al. (1959) reported on how the relative value on a weight basis of carotene and vitamin A for lambs and pigs varies

with the level of intake. With increasing intakes of carotene or vitamin A, plasma vitamin A concentration increased at essentially constant rates.

Later Thompson et al. (1947, 1949 a,b) observed that vitamin A was not found in the intestine before the entrance of the common bile duct, or in the large intestine. The increase of vitamin A in pigs which occur in the lymph after dosing with carotene was accompanied by an increase in yellow color, but it could not be clearly established whether the pigment was unchanged carotene or some derivative. No carotene appeared after dosing in either the portal or systemic blood of pigs. An increase in vitamin A ester preceded an increase of vitamin A alcohol in both circulations.

From all available evidence, it appears that the small intestine is the most common site for the conversion of carotene but that the liver and probably other tissues may be alternative sites. The quantity of provitamin converted in a given time is limited by factors which are not yet understood, but the conversion starts very promptly after a dose of provitamin has been administered.

Vitamin A Requirements of Swine

Vitamin A and carotene requirements of cattle, sheep and swine have been reported by Guilbert et al. (1937) and were found to be 25 to 30 mcg. of carotene or 6 to 8 mcg. of vitamin A daily per kilogram body weight. Excellent growth occurred at these levels, yet storage

after extended period was meager. These requirements support the hypothesis proposed by Guilbert and Hart (1935) that vitamin A requirement is directly related to body weight rather than to energy requirement. Dunlop (1935) also found that the minimum requirement for swine was within this range. Later Guilbert et al. (1940) reported the minimum requirement as the lowest level per unit of body weight that prevented any detectable degree of nyctalopia under light conditions. The ratio of the relative efficiencies of vitamin A and carotene widened with increasing levels of intake. A summary of the recommendations is shown in Table 1.

Sheffy et al. (1954) reported that supplementation of 6 mcg. of vitamin A acetate per kg. body weight to the ration of baby pigs, which had been on a 4-week depletion period since birth, was found to be adequate to provide good growth and prevent the appearance of gross deficiency symptoms. A level of 12 mcg. per kg. body weight restored initial blood plasma levels of the vitamin. Supplementation with 18 mcg. was considered to be the minimum requirement to provide a trace of vitamin A storage in the liver.

Nelson et al. (1962) found that under experimental conditions, the minimum vitamin A requirement for pigs necessary to produce normal plasma vitamin A and some liver storage appeared to be between 8 and 16 mcg. of vitamin A per pound of live-weight per day.

Dunlop (1935) showed that the amount of vitamin A necessary in the diet to keep the animals' reserves at their original level was approximately 60 mcg. carotene per 100 lb. of feed. Or alternatively, the vitamin A requirement of a 100 lb. pig for maintenance

and normal growth (1.3 lb. per day) is 4 mcg. of carotene per day.

The minimum requirements of young pigs, which were depleted of their vitamin A reserves, for purified carotene (80-85 per cent beta-carotene) in cottonseed oil, were reported by Hentges et al. (1952). To restore initial blood plasma vitamin A levels and provide some liver storage of vitamin A, 25 mcg. of purified carotene per kilogram body weight was shown to be the minimum daily requirement. The feeding of 17.5 mcg. per kilogram body weight did not permit liver storage of vitamin A, but was sufficient to restore initial plasma vitamin A levels under the environmental conditions used in one experiment. To overcome gross deficiency symptoms and to promote good average daily weight gains, 10 mcg. of purified protein per kilogram body weight daily were sufficient.

It has been shown that in all animals the requirement for carotene, expressed in International Units, was more than twice those for vitamin A.

The vitamin A requirements of swine have been established by the National Research Council (1959, 1964) and are shown in Table 2.

The Absorption of Carotene and Vitamin A

There are many factors, both chemical and physiological, which influence the absorption of vitamin A and carotene. Vitamin A and its esters, are much more readily soluble in oils and fats than

TABLE 1

Summary of data on Minimum Vitamin A and Carotene Requirements of Swine

	Daily Vitamin A and Carotene Requirements		
	Mcg./Kg. B.W.	I.U./Kg. B.W.	I.U./100 lb. B.W.
Carotene	25-39	42-65	1900-2950
Vitamin A	4.4-5.7	18-24	810-1080

TABLE 2

N.R.C. Requirements for Carotene and Vitamin A of Growing and Finishing Pigs

(Amounts per animal per day)

	Growing Pigs		Finishing Pigs Meat Type		
Live-weight, lb.	25	50	75	125	175
Carotene, mg. [a]	4.0	4.4	6.2	8.0	9.4
Vitamin A, I.U.[a]	2,000	2,200	3,100	4,000	4,700

[a] Carotene and vitamin A values based on 1 mg. beta-carotene equal 500 I.U. of biologically active vitamin A. Vitamin A requirements can be met by either carotene or vitamin A.

carotene. This difference at least partially explains why carotene is usually absorbed less efficiently than vitamin A. (Drummond et al., 1935). In many animals carotene undergoes conversion to vitamin A before passing through the intestinal wall. The question of the absorption of carotene cannot be clearly separated from its conversion. In some circumstances the limiting factor in the absorption of carotene may not depend on its solubility, but on the adequacy of the enzyme systems concerned in their conversion.

Other important factors to be considered are; the influence of emulsifying agents which affect the degree of dispersal of dietary fat, oxydizing agents and antioxidants which affect the stability of its constituents. The absorption of vitamin A will depend on whether it is given as the free alcohol or as its esters. Also the ability of the species or individual to absorb fat, or to digest foods containing the vitamin or its provitamins.

The rate of absorption of vitamin A and carotene from different levels of the gastrointestinal tracts was studied by Barrick et al. (1948). They observed that there was no marked change in the blood vitamin A or carotene level as a result of administering vitamin A in caecum or colon. The change in the vitamin A content of the blood was much slower and less pronounced following the administration of carotene than in the case of vitamin A. Only small amounts of carotene were found in blood following the administration of massive doses of carotene. Vitamin A given orally was absorbed at about the same rate as that placed in the rumen.

Following injection of vitamin A into the small intestine, the vitamin A level of the blood increased sooner than when it was given in the rumen or administered orally. However, it did not reach as high a peak and was not maintained at an increased level for as long a period.

Drummond et al. (1935) demonstrated an increased concentration of vitamin A in lymph collected from the thoracic duct after oral administration of the vitamin. Vitamin A, in contrast to carotene, was very efficiently absorbed by this route.

Popper and Volk (1944) observed a fluorescence in portal blood. These findings suggested that vitamin A may be absorbed by two different routes as described for fats by Frazer (1946). Vitamin A could be absorbed through the intestinal wall and into the portal blood, or it could be accomplished through the lacteals into the lymphatic system.

Eden and Sellers (1949) have shown that in ruminants and rats, vitamin A is absorbed through the intestinal lymph. The lymph draining the small intestine seems to be the main pathway by which the vitamin A reaches the general circulation. In cattle, sheep and rats, the lymph or lymph glands of the intestine contained considerably more vitamin A after the animals had been dosed with vitamin A than when no vitamin A was given.

The major part of the absorption seemed to take place in the upper part of the intestines. They have been unable to obtain any conclusive evidence that vitamin A is carried from the intestine by

the portal blood. Alexander and Goodwin (1950) observed that the oral administration of carotene to conscious rats with the intestinal lymphatic vessel cannulated, resulted in a marked increase in the vitamin A content of the lymph, clearly indicating the conversion of carotene into vitamin A in either the intestine or intestinal wall. No carotene was observed in the lymph during the experiments.

Thompson et al. (1949a, 1949b) found in pigs that after they had been fed a meal of carotene in oil, little or no carotene could be detected in the systemic or portal blood. However, vitamin A ester appeared in increased quantities in the blood within 2 hours after the carotene meal. An increase in the alcohol form followed 2 hours later. Vitamin A, exclusively in the ester form, appeared within 75 minutes in the lymph from the duct draining the mesenteric lymphatics and in the wall and contents of the small intestines. They also showed that when beta-carotene was given by mouth to vitamin A deficient rats, vitamin A appeared within 15 minutes in the walls and contents of the small intestine, whereas the first increase in blood and in the liver did not occur before 45-60 minutes had elapsed. The rapid appearance of vitamin A in the small intestine after a meal of carotene was also demonstrated in normal pigs. It was concluded that the small intestine is the site of conversion of carotene to vitamin A and that the lymphatic system is a route of transport of the vitamin so formed. This is in agreement with the work of Swick et al. (1949, 1952) in the pig. Thompson et al. (1950) stated that in pigs, vitamin A, exclusively as ester, appeared in

high concentration (up to 32 I.U. per ml.) in the lymph within 1 to 2 hours after a meal of carotene or vitamin A. Only traces of carotene were found at any time in the systemic or portal blood of pigs. Kon and Thompson (1951) concluded that beta-carotene is transformed in the intestine to vitamin A ester and then it is carried by the lymph system to the blood stream and finally to the liver.

In vivo study with rats on the mode of absorption of vitamin A esters, Mahadevan et al. (1963) suggested that all esters of vitamin A are hydrolyzed and resynthesized during absorption. In vitro studies showed that prior hydrolysis of vitamin A esters is essential for the absorption and only vitamin A alcohol crosses the mucosa-cell membrane to be re-esterified in the cell, preferably as the palmitate, which in turn is released into the cytoplasm.

Absorption and transport of vitamin A can be summarized as follows: dietary retinyl ester is hydrolyzed in the lumen of the intestine before passage across the mucosa cell wall. Retinol from dietary sources or resulting from the hydrolysis of dietary retinyl ester, passes the mucosa cell wall, and is re-esterified inside the cell, preferentially with palmitic acid. Retinyl palmitate in chylomicrons travels through the lymphatic system, via the thoracic duct, to the blood stream, and is stored in the liver. Stored retinyl ester is hydrolyzed there by a liver enzyme; free retinol then travels via the blood stream to the tissues where a metabolic requirement exists. Retinol is mobilized from the liver and its level in

blood is maintained, even on a diet without vitamin A, until all liver reserves are exhausted (Oswald, 1966).

Liver and Plasma Content

The liver plays an important role under normal conditions of nutrition in serving as the main store house for the vitamin A reserves. The rat, with a life span of some three years, can accumulate enough vitamin in its liver to last, if economically used, for a century (Davies and Moore, 1935). The presence of a reserve does allow the animal to survive during deprivation and that the time of survival roughly depends on the magnitude of the reserve (Moore, 1957).

The quantities of vitamin A accumulated by mammals and stored in their livers, vary widely between species and also between individuals within the same specie. Pigs had lower reserves than herbivorous animals.

Age, sex, season and especially food supply are factors which influence vitamin A content.

Davis and Madsen (1941) reported the vitamin A content of blood plasma to be closely related to its carotene content. However, vitamin A values tend to reach a stable level and do not increase proportionally with increasing carotene intake.

Church et al. (1954) reported highly significant increases in plasma vitamin A levels of wethers after intravenous injections of a solubilized, aqueous carotene preparation. However, there was no

significant difference in the plasma vitamin A level of calves after a similar carotene injection. Their data indicated a difference between cattle and sheep in their ability to convert intravenously injected carotene to vitamin A.

Ganguly and Krinsky (1953) were unable to show any relationship between plasma and liver vitamin A alcohol of rats. Despite wide variations in the concentration of vitamin A alcohol in the liver, the plasma level remained relatively constant.

Grummer et al. (1948) found that pigs are born with a low plasma vitamin A level (0.1 mcg. per 1 cc.). The vitamin A concentrations increased after birth and reached an average maximum of 0.34 mcg. per cc. between the second and seventh days. After the first week there was a general decline in the plasma A levels until the sixth week, followed by a rise until the time of weaning, when a more drastic drop occurred. The vitamin A level after weaning decreased markedly and fluctuated between 0.1 and 0.2 mcg. per cc. until the pigs were 4 months of age.

Baumann et al. (1934) found 95 per cent of the total vitamin A of the rat stored in the liver. Reserves of vitamin A depleted at the rate of 7 to 18 blue units (about 4.2 to 10.8 I.U.) daily, depending on the amount stored. When equal amounts of vitamin A were fed to animals in various stages of depletion, the amount stored was inversely proportional to the state of depletion.

Dann (1932) found liver stores of vitamin A for young rats were greater at weaning than at birth but also more variable.

Davies and Moore (1935) showed that adult rats, when fed massive doses of vitamin A concentrate, built up liver reserves of about 18,000 B.U. (about 10,400 I.U.) per gram. When these rats were placed on a diet free of vitamin A, they rapidly eliminated vitamin A from the liver. After 12 weeks, the mean vitamin A reserves had fallen to 400 B.U. per gram. This level appeared to be the stable storage level for these animals.

Frey et al. (1947) reported in cattle that vitamin A did not exert a sparing action on hepatic stores of carotene. Blood stores of carotene were depleted sooner than were hepatic stores. A rapid decrease in hepatic stores of vitamin A and carotene occurred on a fattening ration consistent with good feeding practice. The depletion rate of hepatic stores of vitamin A and carotene diminished with decreasing hepatic stores of either two constituents. Hepatic reserves of vitamin A were found to be more nearly depleted than were hepatic reserves of carotene. It was found that hepatic reserves of carotene are maintained in direct proportion to the carotene intake. An increasing rate of loss of hepatic reserves of vitamin A occurred with decreasing carotene intake.

Guilbert and Hart (1935) reported the total storage of vitamin A and carotene in the liver and body fat of cows 2 to 18 years old, with access to green feed in abundance throughout life, was estimated to be about 0.6 to 0.7 grams in the younger animals and up to 3.6 grams in aged cows. From 67 to 93 per cent of the storage was primarily in the form of vitamin A, whereas carotene predominated in

the fat. Animals on a carotenoid deficient ration indicated a daily withdrawal of 9 to 11 mcg. per kg. live weight.

Moore and Payne (1942) reported values averaging 22 I.U. of vitamin A per gram of liver for pigs from 6 to 8 months of age. Hentges et al. (1952) observed in pigs that the plasma vitamin A content appeared to be remained within normal range until the liver reserves of vitamin A are almost depleted.

Hemoglobin and Hematocrit

Wintrobe and Shumacker (1936) showed that the hemoglobin and hematocrit of the mammalian fetus and new born pigs were at first very low when compared with those of adults of the same species. As the fetus developed, the amount of hemoglobin and hematocrit increased. Wintrobe (1951) listed the normal hemoglobin and hematocrit values of pigs as 15 gram per 100 ml. and 46.3 per cent of blood, respectively.

Albritton (1952) reported the blood hemoglobin value for 1-2 hour pigs, 11.8 (11.4-12); 1-10 days, 8.1 (5.4-10.1) and adult females 13.8 gram per 100 ml. of blood. The hematocrit value was 41.5 per cent with a range from 30-53 per cent. He also listed the hematocrit values for pigs 1 to 12 hours, 39.6 per cent (39-40 per cent), 1-10 days, 25.0 per cent (18-36 per cent) and adult females 40.8 per cent.

Gardiner et al. (1953) found a postnatal decline in hemoglobin twice as great in litters on concrete floor as in those on ground and pasture during the first week of life. Hematocrit values were

correlated with the hemoglobin values. They listed the hematocrit values for 1 day old, 38.1 per cent, 8 days old, 30.0 per cent and 15 days old, 34.9 per cent.

Barber (1955) observed a hemoglobin decline in indoor-reared pigs which persisted through the seventh week. Outdoor-raised pigs did not exhibit a similar fall in hemoglobin.

Swenson et al. (1958) found a consistent hemoglobin range of 9.2 to 15.3 gram per 100 ml. blood for Durocs and attributed a range of 8.1 to 12.4 gram per 100 ml. blood for Hampshires to the maternal ration during gestation. The hematocrit value for thirty-five Duroc pigs ranging in age from 36 hours to 8 weeks of age were slightly higher than twelve Hampshires of the same ages.

Miller et al. (1961) established hemoglobin and hematocrit determinations for 1802 male and 1876 female swine from birth to 2 years of age. The values ranged from 9.2 $\pm$ 0.1 to 13.9 $\pm$ 0.2 gram per 100 ml. for hemoglobin and 30.0 $\pm$ 0.5 to 44.2 $\pm$ 0.9 per cent of blood for hematocrit. The changes in values of blood hemoglobin and hematocrit which occur throughout the life of swine are relatively parallel. Values for the newborn pig are similar to those found in adult swine. By three days of age a 25 per cent reduction in hemoglobin and hematocrit has occurred. This reduction has been observed by Talbot and Swenson (1963) who reported hemoglobin and hematocrit levels reduced to 6.7 $\pm$ 1.3 gram per 100 ml. and 19.4 $\pm$ 3.1 per cent, respectively, in the second week and remained at about

that level through the fourth week. They also established the normal hemoglobin and hematocrit levels for 31 pigs ranging in age from 6-18 hours (11.2 ± 2.4 gram per 100 ml. and 31.8 ± 5.3 per cent) to eight weeks (10.6 ± 2.0 gram per 100 ml. and 31.6 ± 6.4).

Nitrate Toxicity

The term "nitrate toxicity," as commonly used, is actually "nitrite toxicity" and is produced following the reduction of nitrate to nitrite either in the foodstuff or within the alimentary tract. Nitrite converts the hemoglobin into methemoglobin, which is unable to act as an oxygen carrier. If this change is sufficiently complete animals may die of tissue anoxia; symptoms are seen when about 30 per cent of the hemoglobin is converted into methemoglobin (Whitehead and Moxon, 1952; Garner, 1963).

The toxicity of nitrite is not restricted to the conversion of hemoglobin to methemoglobin but is involved in many other processes. The oxidative destruction of blood and tissue vitamin A and carotene is among the toxic actions of nitrite (Smith, 1961).

Davidson et al. (1941) found when a large proportion of the hemoglobin has been changed to methemoglobin, the affected animals shows typical symptoms of poisoning, rapid acceleration of pulse, shortened quick respiration, trembling of certain muscles, weakness, staggering gait, apparent blindness in some cases, and death from asphyxiation since methemoglobin does not readily yield oxygen to the

tissues of the body. It was shown that nitrite was an intermediate in the conversion of nitrate to ammonia (Lewis, 1951) and this was verified by Wang et al. (1961) in experiments employing N^{15} -labelled nitrate.

Muhrer et al. (1955) and Case (1957) observed that vitamin A deficiency occurred in forages grown during a drought and that these forages contained more nitrate than normal, suggesting that vitamin A deficiency may have been associated with nitrate consumption.

Stewart and Merilan (1958) reported that administration of potassium nitrate to dairy cattle resulted in an increase in methemoglobin, which varied according to the level of KNO_3 intake. Death resulted from an intake of 25 grams KNO_3 per 100 lb. body weight daily for three days.

Kilgore et al. (1959) observed that methemoglobin rises significantly in rabbits and rats with increase in the levels of nitrate ingested either as sodium nitrate or as it occurs naturally in plant food.

Pigs are more susceptible to nitrite poisoning than cattle and sheep, the minimum lethal dose being of the order of 70 to 75 mg. per kg. (32 to 43 mg. per lb.) in the form of sodium nitrite (Garner, 1963).

Tollett et al. (1960) found that methemoglobin level was increased in pigs receiving a ration containing 3.17 per cent nitrate. Emerick et al. (1965) indicated the effect of dietary variations on the severity of nitrate toxicity in sheep was probably due almost

entirely to variation in nitrite accumulation in the digestive tract and not to differences in the rate of methemoglobin reduction per se. They also concluded that young healthy pigs one week of age are no more susceptible to nitrite induced methemoglobinemia than the same pigs at a later age. This is in agreement with the data reported by Seerley et al. (1965).

Effect of Dietary Nitrate and Nitrite on Vitamin A Utilization

Dietary nitrate and nitrite has been implicated as one of the factors for the failure of dietary carotene to sustain in adequate vitamin A nutrition. This includes a possible increase in the vitamin A requirements with present feeding systems. Also it is possible that the destructive mechanism operating may vary depending upon the oxidation state of nitrogen supplied and source of vitamin A fed. A more far-reaching effect may be the alteration of protein enzymes required to convert carotene to vitamin A or the impairment of processes involved in the functional metabolism of vitamin A.

Mitchell et al. (1960) reported vitamin A deficiencies in cattles with poor feedlot performance, although the rations provided carotene in amounts considered adequate by present standards. Such cattle have shown more response to supplementation with performed vitamin A than carotene, indicating an impaired ability to utilize carotene. From this report it is reasonable to speculate that the difficulty involves destruction of carotene in the gastrointestinal

tract of the animal, failure to absorb carotene, or impaired ability to utilize carotene. Whether it relates directly to dietary nitrate or nitrite and other factors involved is not well understood.

Garner et al. (1958b) found that serum nitrate increased with increasing amounts of nitrate to 16 mg. per cent rations.

Koch et al. (1963) studied the effect of different levels of added sodium nitrate and sodium nitrite in growing pigs. They found voluntary feed consumption was decreased as the level of both nitrate and nitrite increased. This will automatically lower vitamin A consumption if the vitamin is fed as a fixed proportion of the ration. But the work of Tollett et al. (1960) did not support the former report.

In a study with rats fed nitrate and nitrite O'Dell et al. (1958, 1960) and Yadav et al. (1962) indicated there was a reduction in the conversion of carotene to vitamin A. They also reported rats which received nitrite as part of the ration, developed vitamin A deficiency.

Pugh et al. (1962), using an in vitro technique, presented evidence that vitamin A or carotene destruction in the presence of nitrite is dependent on pH. Destruction of beta-carotene was very rapid at a pH of 1-3, followed by relatively little destruction at pH 5-7. Furthermore, Olson et al. (1963) reported that nitrite itself had little or no apparent effect on beta-carotene destruction under neutral or alkaline conditions, but when acid was added, the destruction was very rapid. They also reported that addition of the

nitrite to abomasum juice (pH 3) caused almost complete destruction of beta-carotene and suggested that this nitrite effect might occur in monogastric animals when carotene and nitrites are administered orally. Robert and Sell (1963) suggested that dietary nitrite did not enhance vitamin A destruction in the sheep. But in the presence of nitrite, vitamin A is destroyed in the ventriculus area of the digestive tract of the chick, where the pH is approximately 4.

Effect of Nitrate and Nitrite on Liver Vitamin A Storage

The high nitrate content of forages has been suggested as a factor responsible for precipitating vitamin A deficiencies in livestock receiving levels of carotene thought to be adequate (Garner, 1958a). For this purpose liver storage of vitamin A is a better indication of the vitamin A status of an animal than plasma vitamin A and carotene, so the effect of nitrate on vitamin A in this organ has been studied quite extensively. Carotene is not stored in significant quantities in the liver.

In a study with rats and swine Garner et al. (1958b) found that the feeding of nitrate and nitrite resulted in a depletion of liver vitamin A. The feeding of 0.3 per cent of potassium nitrate was reported by O'Dell et al. (1960) to cause an increased rate of depletion of vitamin A stores in the liver of rats. Smith et al. (1961) presented data showing that a ration containing 0.8 per cent KNO_2 reduced liver vitamin A levels and growth of rats and this "chronic nitrite toxicity" could be largely overcome by supplementing the ration with vitamin A or carotene.

Goodrich et al. (1962, 1964) reported that in sheep liver vitamin A stores were significantly lowered by feeding rations containing 3 per cent sodium nitrate with or without the addition of vitamin A. Holst et al. (1961) found that liver vitamin A was low in sheep fed nitrite with the levels varying from 1 per cent initially to 0.75 per cent for the final month. Hale et al. (1961) found that a high concentrate ration (71.3 per cent T.D.N.) caused a significantly ($P < 0.05$) larger depletion of liver vitamin A in steers than did a low concentrate ration (54.3 per cent T.D.N.). When 1 per cent KNO_3 was added, the same trend occurred, however, the difference was not significant. Average liver vitamin A values were reduced from 38 mcg. to 6 mcg. in 56 days. Jordan et al. (1961, 1963) found that feeder steers were depleted of liver vitamin A stores when wintered on corn silages containing carotenes in quantity considerably in excess of supposed needs. The effect was notably exaggerated by silages containing nitrate accumulated in the forage or by a factor associated with nitrate accumulation. Liver stores of vitamin A were depleted more rapidly on a "high nitrate" silage than on a "control" silage. This was in agreement with the report of Weichenthal et al. (1961, 1963) that adding 1 per cent sodium nitrate to a high-grain ration of yearling steers did not affect the vitamin A and carotene content of the liver. Also the reports of Smith et al. (1962) and Jones et al. (1966) found that the addition of KNO_3 as 1-2 per cent of dietary dry matter did not significantly effect liver vitamin A stores.

Other workers (Sokolowski et al. 1961; Cline et al. 1962, 1963) working with sheep were in agreement with the above reports. They found the addition of potassium nitrate from 1 to 4 per cent of air dry feed had no apparent effect on liver storage of vitamin A. But Hatfield et al. (1961) noted a depression in liver vitamin A levels in sheep when high nitrate silage was fed.

Emerick and Olson (1962) reported that in rats the feeding of nitrite, but not nitrate, resulted in a reduction in the amount of vitamin A stored in the liver when vitamin A was administered orally, but did not alter the vitamin when it was injected under the skin. Both nitrate and nitrite significantly lowered liver storage of vitamin A from carotene with the greatest effect resulting from nitrite. They suggested that the action of nitrite in reducing liver storage of the orally administered vitamin A was the result of either a decrease in absorption of the vitamin from the intestine or an increase in its destruction within the digestive tract. Sell and Roberts (1963) studied the effects of 0.4 per cent dietary nitrite (KNO_2) in chicks. They found liver vitamin A levels were low when fed the various levels of vitamin A or carotene along with nitrite. However, when high levels of vitamin A were injected, which were equivalent to levels fed, liver vitamin A concentration was relatively high in nitrite-fed chicks.

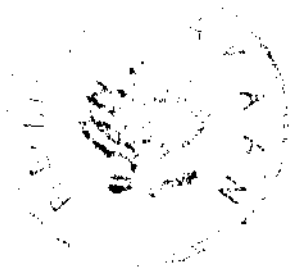
Effects of Nitrate on Performance

Bradley et al. (1940) fed cattle over a two-month period with prolonged sublethal dosages of nitrate and found no loss in weight or other abnormalities. Addition of 1 per cent potassium or sodium nitrate to grain, reduced feed consumption and rate of gain of fattening cattle (Hale et al., 1961; Weichenthal et al., 1961) but in the other experiments additions of 1 to 2 per cent potassium nitrate (dry matter basis) to corn silage or hay did not reduce gains of cattle, nor did additions of 4 per cent potassium nitrate to corn silage affect gains of sheep (Smith et al., 1962). In other experiments, in which fattening lambs were fed up to 4 per cent potassium nitrate in mixed rations that were high in concentrate, rates of gain were either not affected (Cline et al., 1962) or were reduced (Sokolowski et al., 1961). In the latter experiment, increasing the sulfur content of the diet nullified the effect of nitrate on rate of gain, but the significance of this observation is not clear. Hatfield and Smith (1963) reported that the feeding of potassium nitrate at levels increased with the time up to 5 per cent of the diet reduced gains in lambs fed soybean meal but increased gains in lambs fed urea.

Studies of nitrate effects on the growth of swine, Tollett et al. (1960) reported the addition of 1.84 per cent or more of nitrate to the ration significantly depressed gains. In all cases regardless of vitamin A level, the growth of pigs receiving 3.17 per cent nitrate was significantly depressed. This is in agreement

with the report of Koch et al. (1963) that the effect of different levels of added sodium nitrate (from 1 to 5 per cent) and sodium nitrite (0.3 and 0.5 per cent) depressed gains.

In nitrate feeding experiments with swine, where feed consumption was reported, rate of gain has not been reduced unless there was also a reduction in feed consumption. Animals seem to accept a higher percentage of nitrate in forage than in grain mixtures.



CHAPTER III

EXPERIMENTAL PROCEDURE

Three trials were conducted during winter, spring and early summer seasons to study the effects of different levels of potassium nitrate and potassium nitrite added to the ration on the depletion of liver vitamin A stores, plasma vitamin A, methemoglobin, hemoglobin, hematocrit levels and the performance of the pigs.

Trial I

Fifty Yorkshire x Hampshire crossbred pigs were allotted by weight and sex to five ration treatments with two replicate lots each which weighed an average of 56 pounds. The pigs were maintained on a basal ration until the removal of one pig from each pen gave an average weight per treatment of 100 pounds. By receiving a balanced ration the pigs were considered to have adequate liver vitamin A stores. Four pigs were randomly selected for sacrifice from the ten pigs removed when lot weights were even. The liver samples were collected for vitamin A analysis to serve as an initial vitamin A standard. Two pigs from each lot with approximately equal weights were used for blood collection until the termination of the experiment. This trial covered the period of time from November 4, 1965 to February 2, 1966.

The pigs were confined to 4' by 10' sections of housing with an outside runway of a similar dimension. A self-feeder and an automatic waterer were provided for each lot.

The pigs were fed a corn-soybean meal ration which was formulated to contain 16 per cent crude protein until they reached an average weight of 75 pounds. Afterward they were fed a 14 per cent crude protein ration to 125 pounds and finally a 12 per cent crude protein until market weight. At this time the trial was terminated.

The rations fed to the pigs are presented in Table 3. It was considered to be adequate in all nutrients with the exception of vitamin A activity and it was practically devoid in this nutrient. The two most common natural sources of carotene in pigs rations, yellow corn and alfalfa meal, were eliminated and white corn was used as the main energy source. Meat and bone scrap which contains some vitamin A also eliminated.

The amount of nitrate from KNO_3 or nitrite from KNO_2, in per cent, added to the ration of each respective treatment is shown in Table 4.

The pens were cleaned every day, at which time the pigs were observed for vitamin A deficiency symptoms.

The pigs were weighed every two weeks and feed consumption weights were taken at this time.

Blood samples were collected from the anterior vena cava as described by Carle and Dewhirst (1942) each two weeks when the pigs were weighed. They were taken with a glass hypodermic syringe

TABLE 3

Percent Composition of Basal Rations

Ingredients	Per cent Crude Protein		
	16[a]	14[b]	12[c]
Ground white corn	76.90	82.50	88.20
Soybean meal, 44%	21.10	15.50	9.80
Ground limestone	0.70	0.70	0.70
Steamed bone meal	0.50	0.50	0.50
Iodized salt	0.50	0.50	0.50
Trace mineral mixture[d]	0.20	0.20	0.20
B-vitamin supplement[e]	0.10	0.10	0.10
Vitamin B_{12} supplement[f]	0.05	0.05	0.05
Vitamin D_2 supplement[g]	0.15	0.15	0.15
Antibiotic premix[h]	0.04	0.04	0.04
Total	100.00	100.00	100.00

[a]16% ration contained the following analysis: protein, 15.97%; fat, 3.02%; and fiber, 3.17%.

[b]14% ration contained the following analysis: protein, 14.00%; fat, 3.34%; and fiber, 2.74%.

[c]12% ration contained the following analysis: protein, 11.98%; fat, 3.52%; and fiber, 2.54%.

TABLE 3 - Continued

[d]Composition of 1 lb. of the trace mineral mixture was as follows: manganese, 45.339 grams (min.); iron, 45.359 grams (min.); calcium, 45.359 grams (min.); calcium, 54.431 grams (max.); copper, 4.536 grams (min.); zinc, 45.359 grams (min.); iodine, 1.361 grams (min.); cobalt, 0.454 grams (min.).

[e]One pound of B-vitamin supplement supplied not less than 2,000 milligrams riboflavin, 4,000 milligrams pantothenic acid, 9,000 milligrams niacin and 10,000 milligrams choline chloride.

[f]Vitamin B_{12} supplement contained 20 milligrams of vitamin B_{12} activity per pound.

[g]Vitamin D_2 supplement contained 26,450 I.U. of vitamin D_2 per gram.

[h]Antibiotic premix contained feed grade bacitracin methylene disalicylate which was equivalent to 25.0 grams bacitracin (master standard) for each pound of fortracin-25 (minimum levels).

TABLE 4

Levels of Potassium Nitrite (NO_2) and Potassium Nitrate (NO_3) for Trial I

Treatment	NO_2 %	NO_3 %
I	---	---
II	0.075	---
III	0.300	---
IV	---	0.75
V	---	3.00

and a 20 gauge, 3 inch needle. The pigs were restrained with a chain snare on the snout while the blood sample was being drawn. Raising the snout high enough to nearly raise the front feet of the pigs off the ground seemed to improve the ease of obtaining the sample.

Methemoglobin, hemoglobin and hematocrit were analyzed immediately after the blood was drawn.

A small portion of the central lobe of livers from the same pigs from which blood was drawn were removed at the termination trial. These liver samples were then immediately stored at -20°C for later vitamin A analysis.

Analysis of Plasma Samples

After being collected, blood samples were immediately placed in centrifuged tubes, mixed and then placed in a covered container to protect from light. If the plasma could not be removed from the samples, they were immediately refrigerated until this could be done. The blood was centrifuged for 12 minutes. The plasma was then removed and placed in vials or tubes and frozen until they could be analyzed, usually within one month. Vitamin A was extracted by the method of Kimble (1934) with a few modifications. The total vitamin A was determined with the color developed by the trifluoroacetic acid method, described by Dugan *et al.* (1964). Trifluoroacetic acid reagent produces a spectrally identical colored species to that formed by $SbCl_3$, the Carr-Price reagent. Only the

analysis with trifluoroacetic acid retained the advantages of $SbCl_3$ while eliminating some of the disadvantages of the determinations as described by Carr and Price (1926).

The optical density of the blue color formed by the reaction between the trifluoroacetic acid reagent and vitamin A was read immediately (within 10 seconds) at 616 millimicrons with a Bausch and Lomb Spectronic 20 spectrophotometer.

Analysis of Liver Samples

The liver samples were analyzed by the method of Gallup and Hoefer (1946). The total vitamin A was determined with the same method as described for vitamin A in plasma by Dugan et al. (1964).

Analysis of Methemoglobin, Hemoglobin and Hematocrit

Blood samples were immediately placed into the test tubes which contained an anticoagulant. The tubes were then mixed and used for methemoglobin, hemoglobin and hematocrit analysis within one hour.

The methemoglobin determinations were made by the procedure of Evelyn and Malloy (1938). Methemoglobin has a characteristic light absorption at 635 millimicrons. This absorption at 635 millimicrons before and after adding cyanide is a measure of the methemoglobin present.

The hemoglobin determinations were made by using a photoelectric hemoglobin and glucose meter (Lumetron Photoelectric Hemoglobin and Glucose Meter, Model 15, Photovolt Corp., New York, N. Y.).

Hematocrit determinations were made by the use of a microcapillary hematocrit centrifuge (International Microcapillary "Hematocrit" Centrifuge, International Equipment Co., Boston, Mass.). The whole blood was drawn into microcapillary tubes which were sealed and then centrifuged for five minutes at 10,000 rpm.

Trial II

Six purebred Hampshire and thirty-four purebred Yorkshire pigs were allotted to five ration treatments on the basis of breed, sex and weight with replicated lot of four pigs each. The average initial weight was 57 pounds. This trial extended from February 28, 1966, to May 19, 1966.

The pigs were maintained in an 8' by 10' section of indoor-outdoor combination pens, having concrete floors.

All other management practices were the same as described for Trial I, except that in addition to the ration shown in Table 3, the N.R.C. requirement of carotene and vitamin A were also added to provide adequate vitamin A activity.

Dry beta-carotene beadlets (Roche) were provided as the supplemental source of carotene which contained 15 per cent

beta-carotene. The source of supplemental vitamin A was vitamin A palmitate which contained 30,000 I.U. per gram. They were premixed and then added to the basal ration.

Liver samples for the initial vitamin A standard were obtained from four randomly selected pigs that included one Hampshire and three Yorkshire pigs.

The treatments were rations containing the amounts of nitrate in per cent that are presented in Table 5.

TABLE 5

Levels of Carotene, Vitamin A and KNO_3 for Trial II

Treatment	Carotene Equivalent to True Vitamin A I. U.	Vitamin A I. U.	NO_3 %
I	500	---	0.00
II	500	---	0.75
III	500	---	1.50
IV	500	---	3.00
V	---	500	3.00

The analysis of plasma vitamin A, liver vitamin A, methemoglobin, hemoglobin and hematocrit, were the same as described for Trial I.

When the control pigs (Treatment I) reached an average near market weight, the trial was terminated.

Trial III

Seven purebred Hampshire and thirty-three purebred Yorkshire pigs were allotted according to breed, weight and sex, to five ration treatments with replicated lot of four pigs each. The average initial weight was 58 pounds.

An 8' by 10' section of housing with an outside runway of the same dimensions which has a concrete floor was provided for each lot.

All other management practices used in this trial were similar to those of Trial I, except that in addition to the ration shown in Table 3, the N.R.C. requirement of carotene and vitamin A were also provided as described for Trial II.

The amounts of carotene or vitamin A and potassium nitrite (expressed as NO_2) added to each pound of the respective ration treatments are shown in Table 6.

The initial vitamin A standard that was used in this trial was the same as described for Trial II. Criteria for evaluation of the treatments were as described for Trial I. This trial was terminated when the pigs in the control ration (Treatment I) reached market weight.

TABLE 6

Levels of Carotene, Vitamin A and KNO_2 for Trial III

Treatment	Carotene Equivalent to True Vitamin A I. U.	Vitamin A I. U.	NO_2 %
I	500	---	0.000
II	500	---	0.075
III	500	---	0.150
IV	500	---	0.300
V	---	500	0.300

Criteria evaluation for each trial included weight gains, feed consumption and feed efficiency.

To compare average daily gain, average plasma vitamin A, average liver vitamin A stores and average methemoglobin of the controls against each of the other treatment means, Dunnetts test was used as described by Steel and Torrie. Linear regression test (Snedecor, 1956; Steel and Torrie, 1960; Ezekiel and Fox, 1963) was used to obtain a relationship between treatments and average plasma vitamin A and also between treatments and average methemoglobin levels.

CHAPTER IV

RESULT AND DISCUSSION

Trial I

The trial was conducted to study the effects of dietary potassium nitrate (KNO_3) and potassium nitrite (KNO_2) on the depletion of liver vitamin A stores, plasma vitamin A levels, methemoglobin, hemoglobin and hematocrit blood levels and also the performance of the pigs.

Throughout the length of the trial, the general health of the pigs appeared to be normal. However, a bluish discoloration of the mucous membranes and unpigmented areas of the body were observed on pigs receiving Treatment III and V (the higher nitrite and nitrate levels) from the fourth week to the termination of the trial.

A summary of the average daily gain, average amount of feed required per pound of gain and average daily feed consumed, are presented in Table 7. The average daily gains ranged from 1.44 to 1.83 pounds for the pigs receiving Treatments III and I, respectively.

Analysis of variance test (Snedecor, 1956; Steel and Torrie, 1960) of the average daily gain indicated a highly significant difference ($P < .01$) between treatments. Duncan's new multiple-range test (1955) was used for comparisons among treatments. Treatments III and V were significantly different ($P < .01$) from Treatments I and II. Also Treatment III was significantly different ($P < .01$)

TABLE 7

Results of Pig Performance for Trial I

Treatment	Average Initial Weight (Lbs.)	Average Final Weight (Lbs.)	Average Daily Gain (Lbs.)	Feed Per Lb. Gain (Lbs.)	Average Daily Feed Consumption
I (Control	56.5	220.8	1.83	3.94	7.19
II	58.3	221.8	1.82	3.87	7.02
III	56.0	185.8	1.44**	3.76	5.42
IV	55.3	209.3	1.71	3.93	6.72
V	51.9	190.3	1.54**	4.91	7.54

**$P < .01$

from Treatment IV. But Treatment V was only significantly different ($P < .05$) from Treatment IV. To compare the control with the other treatment means, Dunnett's test was used, as described by Steel and Torrie. Treatment III and V were the only ones to show a highly significant difference ($P < .01$) from the control.

The average daily gain was reduced as the levels of nitrate and nitrite in the diet increased. The reduction of the average daily gain is in agreement with the work of Tollett et al. (1960) who reported that young growing pigs fed nitrate additions above 1.13 per cent significantly depressed gains. This is also in agreement with the report of Koch et al. (1963) that the addition of sodium nitrate or nitrite above 3 and 0.3 per cent respectively, depressed the average daily gain of growing pigs.

The average amount of feed required per pound of gain ranged from 3.76 pounds for Treatment III to 4.91 pounds for Treatment V. The control lot required 3.94 pounds of feed per pound of gain. This contrasted with the works of Koch et al. (1963) and Sell and Roberts (1963) who indicated that nitrate and nitrite reduced feed efficiency.

Treatment means of the plasma vitamin A levels for the initial, mid-trial and final collection is summarized in Table 8. The range of the final collection was from 12.6 mcg. of vitamin A per 100 ml. blood for Treatment V to 18.7 mcg. of vitamin A per 100 ml. blood for Treatments II and IV. Treatment III and V had the

TABLE 8

Results of Average Plasma Vitamin A Levels

(mcg. per 100 ml. blood) for Trial I

Treatment	Initial Collection (1st day)	Mid - trial Collection (28th day)	Final Collection (56th day)
I	24.7	15.8	15.9
	(5.24)[a]	(4.77)	(2.01)
II	25.5	21.7	18.7
	(2.35)	(4.78)	(6.36)
III	27.5	17.3	13.7
	(9.35)	(1.18)	(2.82)
IV	31.6	16.9	18.7
	(2.47)	(6.50)	(2.52)
V	30.1	22.2	12.6
	(6.50)	(7.30)	(0.39)

[a]Standard deviations

lowest level of vitamin A, 13.7 and 12.6 mcg. per 100 ml. blood, respectively. However, there are no differences in the final plasma vitamin A levels which could be attributed to treatment effects. This is in agreement with the study of Tollett et al. (1960) who reported that the dietary nitrate did not affect the plasma vitamin A level. This also coincided with the work of Jones et al. (1966) who reported that the addition of 0.75 or 1.25 per cent KNO_3 on a dry matter basis, had no demonstrable effect on plasma vitamin A.

The plasma vitamin A values were shown in Figure 1. There was a rapid decline for all treatments during the first week of the trial. Starting from the sixth week, all treatments showed a gradual decline to the end of the trial. The final plasma vitamin A values for all treatments were lower than the initial values. The lowest levels were found in pigs receiving Treatment III and V, which were supplemented with higher levels of nitrite and nitrate. This is in accord with the work of Koch et al. (1963) that the addition of nitrate or nitrite to the ration of partially vitamin A depleted pigs, will decrease vitamin A level. However, they showed no significant decrease. This was also reported by Goodrich et al. (1962, 1964) and Smith et al. (1962).

At the beginning of the treatment period, liver vitamin A values were obtained from four randomly selected pigs, to serve as a standard for the liver vitamin A. The average value was 48.01 mcg. per gram of liver tissue.

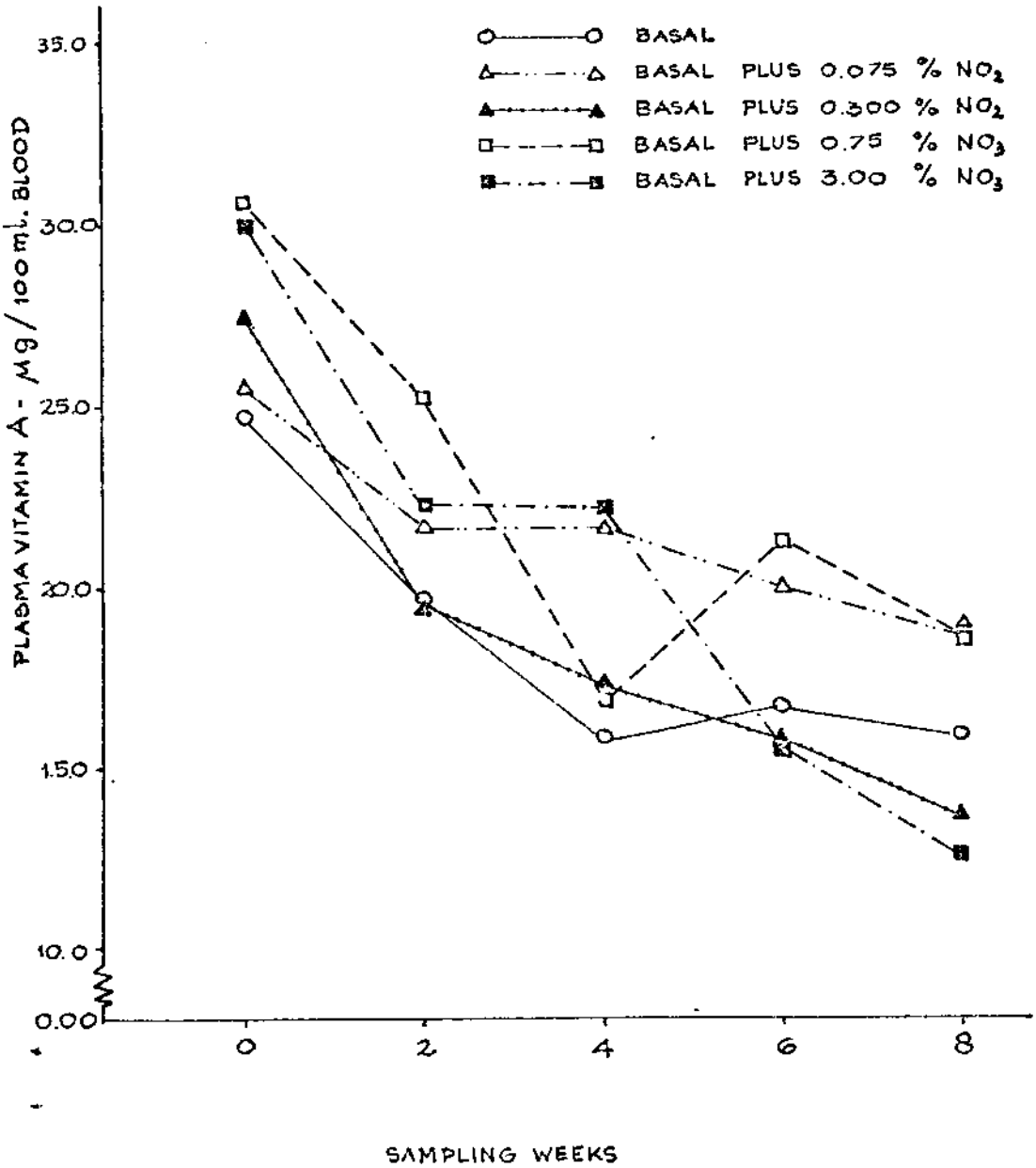


FIG. 1. PLASMA VITAMIN A AT 2-WK INTERVALS FOR TRIAL I

The summary of the average liver stores of vitamin A for each trial is shown in Table 9. The range in Trial I was from 12.8 to 20.17 mcg. per gram of liver tissue for the pigs on Treatments I and IV, respectively.

Analysis of variance test of the average liver vitamin A levels indicated no significant differences between treatments. This is in agreement with the work of Emerick and Olson (1962) who showed that the feeding of 3 per cent sodium nitrate did not alter the liver storage of vitamin A in rats. Also this study is in agreement with other workers who reported that cattle (Hale et al., 1961, 1962a, b; Smith et al., 1962; Weichenthal et al., 1961, 1963) fed 1 per cent of sodium or potassium nitrate and sheep (Cline et al., 1962; Smith et al., 1962, 1963) fed 4 per cent of sodium or potassium nitrate, showed no reduction in the liver vitamin A storage. However, O'Dell et al. (1960) fed 0.3 per cent $NaNO_3$ to rats and Goodrich et al. (1962, 1964) fed 3 per cent $NaNO_3$ to sheep indicated an increased depletion rate of vitamin A stores.

Treatments means for the methemoglobin, hemoglobin and hematocrit levels are presented in Table 10. There are no significant differences between treatments for the final average methemoglobin levels. These results agree with previous observations that feeding 3 per cent of sodium nitrate to sheep with or without vitamin A supplementation did not alter methemoglobin level (Goodrich et al., 1962, 1964). Smith et al., (1962) reported that 4 per cent sodium

TABLE 9

Results of Average Liver Stores of Vitamin A

For Trials I, II and III (mcg. per gram of liver tissue)

Treatment	Starting trial level	Trial I	Trial II	Trial III
		48.01 (10.16)[a]	67.84 (11.45)	67.84 (11.45)
I		12.80 (0.62)	28.05 (18.55)	21.13 (5.86)
II		19.35 (5.86)	22.45 (2.16)	17.23 (7.41)
III		15.64 (3.24)	21.24 (4.00)	11.93 (5.27)
IV		20.17 (6.10)	23.38 (2.78)	13.31 (5.52)
V		14.60 (2.26)	20.14 (6.60)	9.48 (5.43)

[a]Standard deviations

TABLE 10

Results of Average Methemoglobin, Hemoglobin, And Hematocrit for Trial I

Treatment	Sampling Weeks				
	0	2	4	6	8
I					
Methemoglobin[a]	0.42	0.17	0.20	0.05	0.00
Hemoglobin[a]	10.95	11.10	11.58	12.00	11.90
Hematocrit[b]	40.25	40.25	40.50	44.00	44.33
II					
Methemoglobin	0.67	0.38	0.19	0.10	0.04
Hemoglobin	11.83	10.58	11.25	11.58	11.53
Hematocrit	42.50	40.75	40.50	41.25	43.50
III					
Methemoglobin	0.57	2.75	1.00	3.71	1.47
Hemoglobin	10.95	10.93	10.05	10.50	10.13
Hematocrit	40.00	38.00	37.50	41.50	35.33
IV					
Methemoglobin	0.77	0.12	0.28	0.00	0.05
Hemoglobin	11.00	10.65	12.00	12.30	11.13
Hematocrit	40.00	42.67	43.25	45.00	43.50
V					
Methemoglobin	0.32	0.65	0.10	0.00	0.80
Hemoglobin	10.95	11.73	11.58	11.88	11.25
Hematocrit	41.00	42.50	42.00	42.75	42.25

[a]Methemoglobin and Hemoglobin measured in grams per 100 ml. of blood.

[b]Hematocrit measured in per cent of blood.

nitrate did not affect methemoglobin formation in sheep. Also with the feeding of sodium and potassium nitrite (Holst et al., 1961) levels from 0.1 per cent initially to 0.75 per cent for the final month which showed no increase in the methemoglobin level of sheep. This is in contrast with the work of Koch et al., (1963) that the addition of 0.3 to 0.5 per cent of sodium nitrite to non vitamin A deficient and partially vitamin A depleted pigs caused an increase in the methemoglobin level.

The results of the average methemoglobin levels for the initial, mid-trial and final collection periods are shown in Table 11. The range of the final collection was from 0.00 to 1.47 gram per 100 ml. of blood for Treatment I and III, which was the control and the highest nitrite level, respectively.

Methemoglobin values for the entire trial is plotted at two week intervals in Figure 2. The highest peaks of the methemoglobin values, 2.75 and 3.71 grams per 100 ml. of blood were found during the second and the sixth weeks of the trial for Treatment III. The peak remained high, 1.47 gram, at the end of the trial. The pigs on Treatment V had a final level of 0.80 gram per 100 ml. blood.

The average hemoglobin levels were about the same for all treatments (Table 10). The range of the final collection period was from 10.13 to 11.9 grams per 100 ml. of blood for the pigs on Treatments III and I, respectively. This represents a 9.48 per cent difference between the two treatments. These results are supported by Koch et al. (1963) who reported that 0.3 to 0.5 per cent of

TABLE 11

Results of Average Methemoglobin Levels

(mcg. per 100 ml. blood) for Trial I

Treatment	Initial Collection (1st day)	Mid-trial Collection (28th day)	Final Collection (56th day)
I	0.42 (0.50)[a]	0.20 (0.40)	0.00 (0.00)
II	0.67 (0.35	0.19 (0.15)	0.04 (0.08)
III	0.57 (0.44)	1.00 (0.42)	1.47 (0.66)
IV	0.77 (0.53)	0.28 (0.32)	0.05 (0.10)
V	0.32 (0.37)	0.10 (0.20)	0.80 (1.59)

[a]Standard deviations

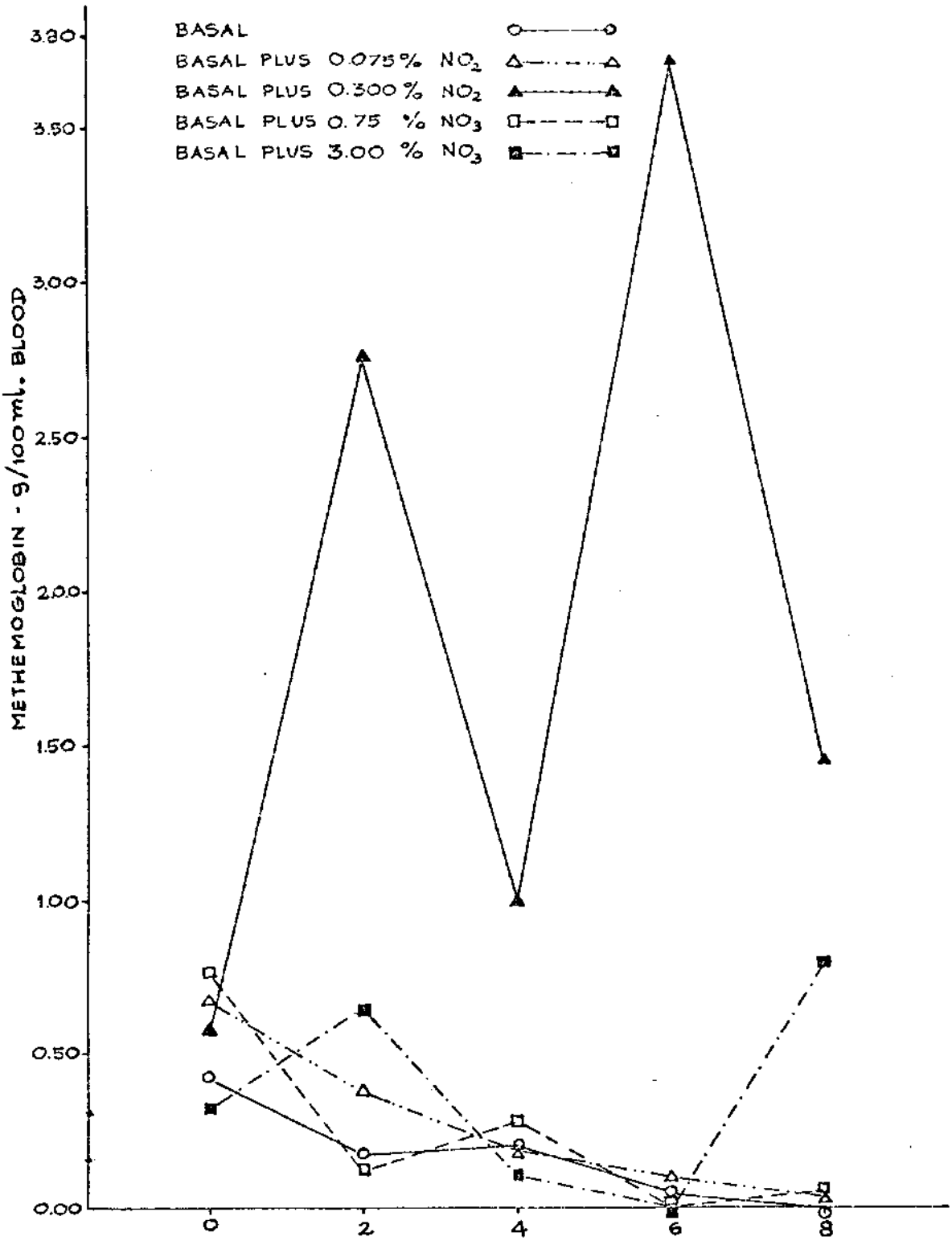


FIG. 2. METHEMOGLOBIN AT 2-WK INTERVALS FOR TRIAL I

sodium nitrate fed to pigs did not effect hemoglobin levels. Jones et al. (1966) made similar observations with cattle.

Treatment means of hemoglobin levels are plotted in Figure 3. The pigs on Treatment I, IV, and V, have higher final levels (11.90, 11.13 and 11.25 gram per 100 ml. blood) than the initial levels (10.95, 11.00 and 10.95 gram per 100 ml. blood). This is in contrast with the final hemoglobin level on Treatment II, especially on Treatment III. They have lower levels, 11.53 and 10.13 gram per 100 ml. blood compared to the initial levels, 11.83 and 10.95 gram per 100 ml. blood, respectively.

The average hematocrit values were shown in Figure 4. The values were quite similar, as summarized in Table 10. The hematocrit levels on Treatment I, II, and V, were similar with some variations during the trial period. But the final hematocrit level on Treatment III was lower, 35.3 per cent of blood compared to the initial level, 40.0 per cent of blood.

The hemoglobin and hematocrit as shown above are within ranges described by Albritton (1952), Swenson et al. (1958) and Miller et al. (1961). It could be postulated that neither nitrate nor nitrite markedly influenced hemoglobin and hematocrit levels.

Trial II

This trial was conducted to study the effect of dietary potassium nitrate (KNO_3) on liver vitamin A depletion, plasma vitamin A levels, methemoglobin, hemoglobin and hematocrit blood levels and performance of the pigs.

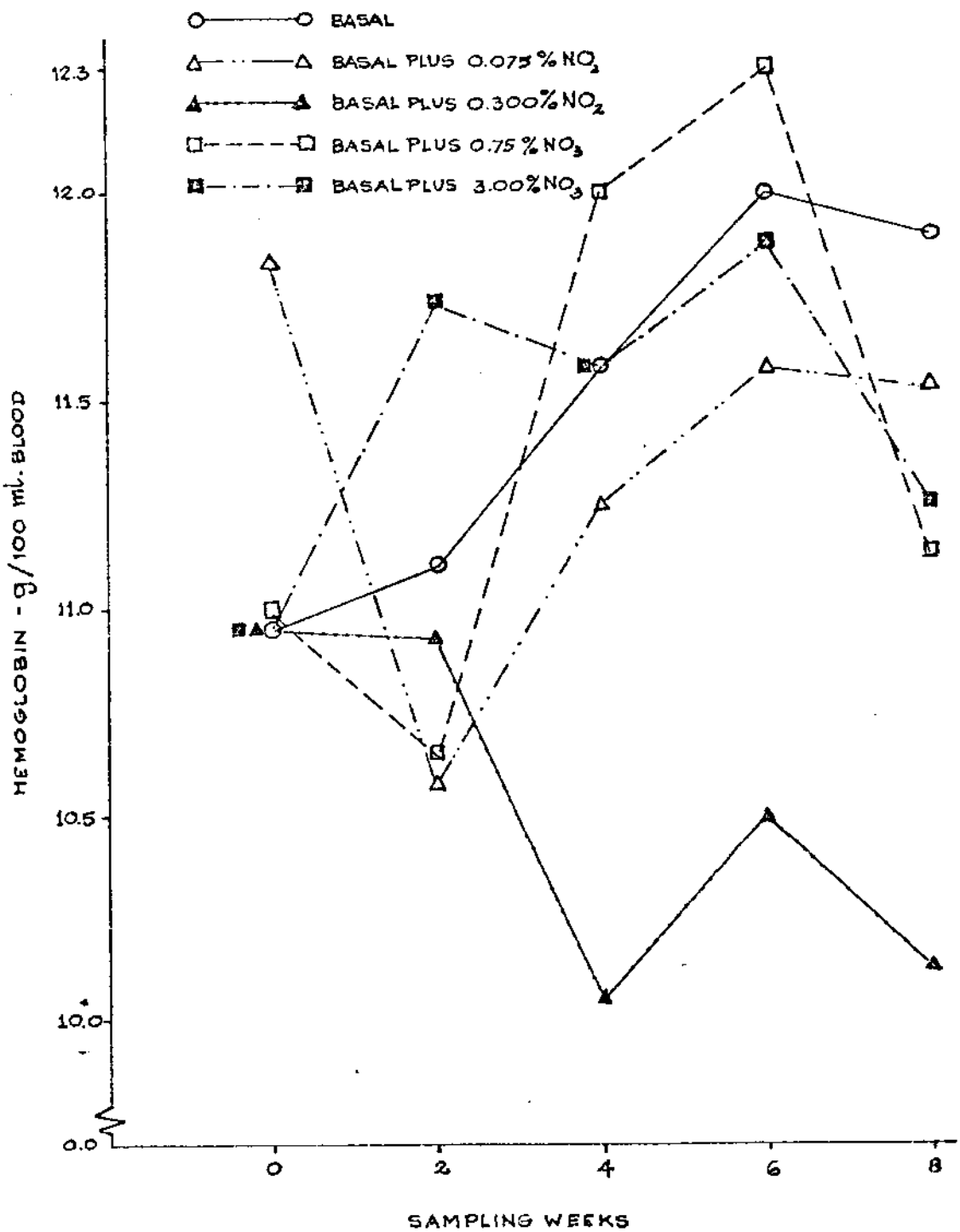


FIG. 3 HEMOGLOBIN FORMATION IN BLOOD OBTAINED FROM PIGS AT 2-WK INTERVALS FOR TRIAL I

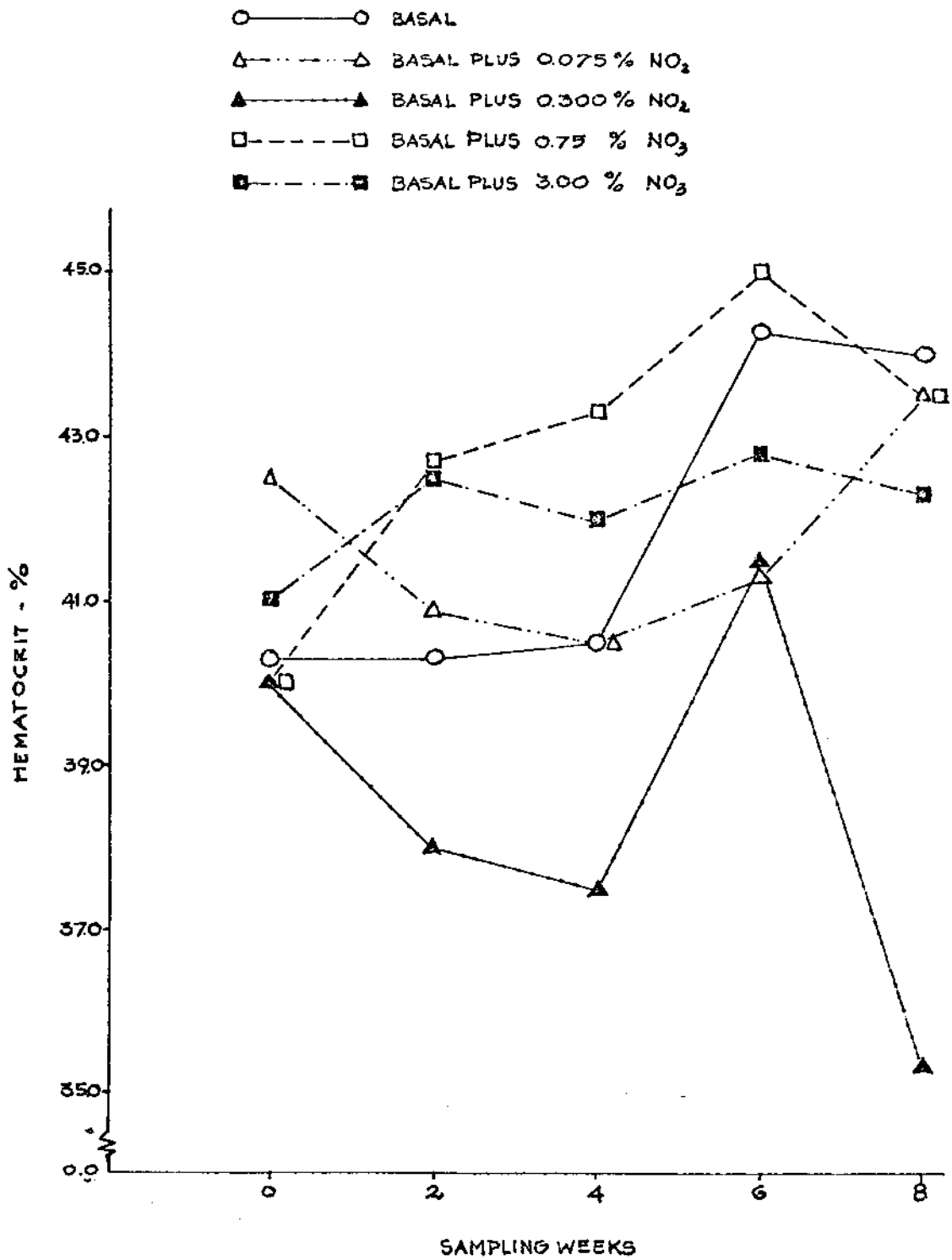


FIG. 4 HEMATOCRIT FORMATION IN BLOOD OBTAINED FROM PIGS AT 2-WK INTERVALS FOR TRIAL I

The general health of the pigs appeared to be normal during the entire trial period. The symptoms observed in this trial were as described for Trial I, on pigs receiving Treatment IV and V (the higher nitrate level) during the last period of the trial. Two pigs were removed from the trial, which were not attributable to treatment effects.

The average daily gain, average amount of feed required per pound of gain and average daily feed consumed, are summarized in Table 12. The average daily gains ranged from 1.20 to 1.55 pounds for the pigs receiving Treatments V and III, respectively. The average amount of feed required per pound of gain increased as the per cent of nitrate in the ration increased. The range was from 3.43 pounds for Treatment II to 4.76 pounds for Treatment V. The control lot required 3.46 pounds of feed per pound of gain. Thus, feed efficiency for all treatments was not markedly reduced by the feeding of nitrate. This contrasted with the reports of Tollett et al. (1960) and Koch et al. (1963) who observed that reduction in feed consumption and rate of gain occurred when the level of nitrate nitrogen exceeded 0.34 per cent of the diet.

Analysis of variance test of the average daily gain indicated a highly significant difference ($P < .01$) between treatments. Treatments II and III were significantly different ($P < .01$) from Treatment IV and V and Treatment IV was not significant from Treatment V. This indicated that the feeding of 3 per cent nitrate receiving vitamin A did not show a significant difference when

TABLE 12

Results of Pig Performance for Trial II

Treatment	Average Initial Weight (Lbs.)	Average Final Weight (Lbs.)	Average Daily Gain (Lbs.)	Feed Per Lb. Gain (Lbs.)	Average Daily Feed Consumption
I (Control)	55.9	192.1	1.54	3.46	5.29
II	57.3	195.2	1.54	3.43	5.29
III	58.2	194.9	1.55	3.92	6.05
IV	57.8	165.4	1.21**	3.97	4.77
V	56.4	164.1	1.20**	4.76	5.81

**$P < .01$

compared to the feeding of 3 per cent nitrate receiving carotene on the average daily gain. Comparing the control with the other treatment means, Treatment IV and V were the only ones to show a highly significant difference ($P < .01$). This is in agreement with the work of Tollett et al. (1960) who reported that young growing pigs fed nitrate above 1.13 per cent depressed gains significantly. This is also in agreement with the report of Koch et al. (1963) that the addition of sodium nitrate above 3 per cent depressed the average daily gain of growing pigs.

A summary of the plasma vitamin A levels for the initial, mid-trial and final collection is shown in Table 13. The range of the final collection was from 17.5 to 23.7 mcg. of vitamin A per 100 ml. blood plasma for Treatment V and I, respectively. Treatment IV and V had the lowest level of vitamin A, 17.7 and 17.5 mcg. per 100 ml. blood, respectively. No difference effect of nitrate - vitamin A ration (Treatment V) on plasma vitamin A levels compared to nitrate-carotene ration (Treatment IV).

The final plasma vitamin A levels failed to indicate any significant difference which could be attributed to treatment effects. This is in agreement with the studies of Tollett et al. (1960), Smith et al. (1962) and Jones et al. (1966) who reported that dietary nitrate did not affect the plasma vitamin A level.

The plasma vitamin A values are shown in Figure 5. There was a decline for all treatments during the first to the sixth (mid - trial) week with the exception of treatment II during the second to

TABLE 13

Results of Average Plasma Vitamin A Levels

(mcg. per 100 ml. blood) for Trial II

Treatment	Initial Collection (1st day)	Mid-trial Collection (42nd day)	Final Collection (80th day)
I	37.5 (9.94)[a]	17.6 (3.02)	23.7 (7.73)
II	22.7 (8.68)	17.7 (1.11)	20.7 (8.63)
III	18.87 (4.34)	21.0 (2.76)	20.5 (7.67)
IV	36.3 (3.81)	17.1 (6.53)	17.7 (3.57)
V	31.7 (5.25)	16.3 (1.27)	17.5 (3.39)

[a]Standard deviations

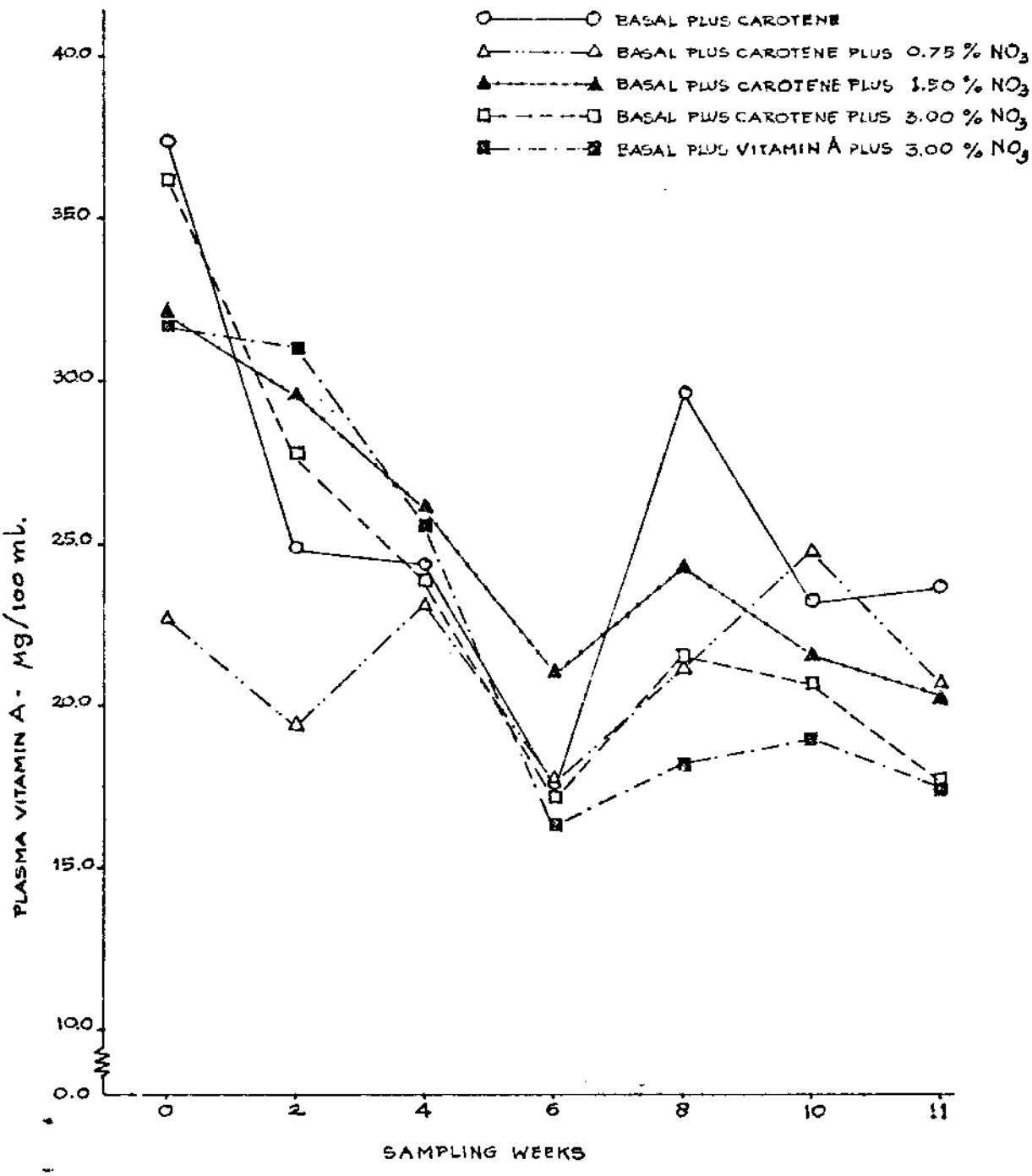


FIG. 5 PLASMA VITAMIN A AT 2-WK INTERVALS FOR TRIAL II

the fourth week. Starting from the sixth week, all treatments showed a gradual increase to the eighth week, with the exception of Treatment III that continued to increase until the tenth week. Treatment II through V showed a gradual decline to the termination of the trial. Treatment I exhibited a slight increase from the tenth week to the end of the trial. The final plasma vitamin A levels for all treatments were lower compared to the initial levels. The lower levels were found in pigs receiving Treatment IV and V, which had the higher nitrate levels. This is in agreement with the work of Koch et al. (1963) and Goodrich et al. (1962, 1964) that the addition of nitrate to the ration decreased plasma vitamin A level. Although they showed no significant decrease.

At the beginning of the treatment period, liver vitamin A values were obtained from four randomly selected pigs, to serve as a standard for liver vitamin A. The average value was 67.84 mcg. per gram of liver tissue. The range of the final average of liver vitamin A stores was from 20.14 to 28.05 mcg. of liver tissue for the pigs on Treatment V and I, respectively.

Liver vitamin A stores were decreased as the nitrate level in the diet increased. However, there were no significant differences between the control and other treatments. Furthermore, nitrate fed to pigs supplemented with vitamin A (Treatment V) tended to reduce liver vitamin A faster when compared to nitrate-fed pigs given supplemental carotene (Treatment IV). Several workers (Cline et al., 1962; Emerick and Olson, 1962; Hale et al., 1961, 1962a,b; Smith et al.,

1962, 1963; Weichenthal et al., 1961, 1963) supported these data when they reported that feeding potassium or sodium nitrate did not reduce liver vitamin A stores. In contrasts, Goodrich et al. (1962, 1964) showed that the addition of nitrate to the ration caused an increased rate of depletion of vitamin A stores.

A summary of the average methemoglobin, hemoglobin and hematocrit values is shown in Table 14. Analysis of variance test of the final average methemoglobin did not indicate any significant difference between treatments. This is in agreement with the other workers (Goodrich et al., 1962, 1964; Holst et al., 1961; Smith et al., 1962; Koch et al., 1963) who indicated that the addition of dietary nitrate did not increase methemoglobin level.

Treatment means of the methemoglobin levels for the initial, mid-trial and final collection period are presented in Table 15. The range of the final collection was from 0.04 to 0.68 gram per 100 ml. of blood for Treatment I and II, respectively. This indicated that methemoglobin level tended to increase as the nitrate level increased.

Methemoglobin formation for the entire treatment is plotted in Figure 6. There was an increase in methemoglobin formation during the first to the second week of the trial. The highest peak, 2.37 gram per 100 ml. blood was obtained at the sixth week on Treatment III, followed by the peak on Treatment II, 1.05 gram per 100 ml. of blood. Final methemoglobin formation on the nitrate - vitamin A ration (Treatment V) had a lower level when compared to the nitrate - carotene ration (Treatment IV), but did not show a significant difference.

TABLE 14

Results of Average Methemoglobin, Hemoglobin, and Hematocrit for Trial II

Treatment	Sampling Weeks						
	0	2	4	6	8	10	11
I							
Methemoglobin[a]	0.29	0.73	0.69	0.60	0.30	0.19	0.40
Hemoglobin[a]	9.98	9.60	11.55	9.80	11.20	10.13	10.73
Hematocrit[b]	37.75	39.00	40.75	40.75	41.75	39.75	42.50
II							
Methemoglobin	0.46	0.60	0.39	1.05	0.54	0.40	0.07
Hemoglobin	9.90	11.77	9.27	9.80	10.70	9.75	10.60
Hematocrit	36.00	43.67	39.00	40.00	38.00	38.00	40.33
III							
Methemoglobin	0.14	0.47	0.65	2.37	0.94	0.72	0.27
Hemoglobin	9.75	10.13	9.75	8.30	10.73	9.90	10.50
Hematocrit	37.00	37.75	39.25	35.80	40.50	38.00	39.25
IV							
Methemoglobin	0.32	0.74	0.38	0.67	0.40	0.37	0.68
Hemoglobin	11.83	10.80	11.35	9.45	10.58	9.15	10.42
Hematocrit	36.00	40.75	39.00	39.75	37.75	37.25	39.00
V							
Methemoglobin	0.48	0.78	0.00	0.75	0.33	0.40	0.32
Hemoglobin	10.70	10.90	10.50	9.10	10.80	9.70	10.30
Hematocrit	37.00	36.67	37.00	36.33	39.33	38.00	38.50

[a]Methemoglobin and Hematocrit measured in gram per 100 ml. of blood.

[b]Hematocrit measured in per cent of blood.

TABLE 15

Results of Average Methemoglobin Levels

(gram per 100 ml. blood) for Trial II

Treatment	Initial Collection (1st day)	Mid-trial Collection (42nd day)	Final Collection (80th day)
I	0.29 (0.37)[a]	0.60 (0.51)	0.04 (0.08)
II	0.46 (0.71)	1.05 (0.64)	0.07 (0.12)
III	0.14 (0.19)	2.37 (0.49)	0.27 (0.24)
IV	0.32 (0.56)	0.67 (0.45)	0.68 (0.89)
V	0.48 (0.22)	0.75 (0.85)	0.32 (0.29)

[a]Standard deviations

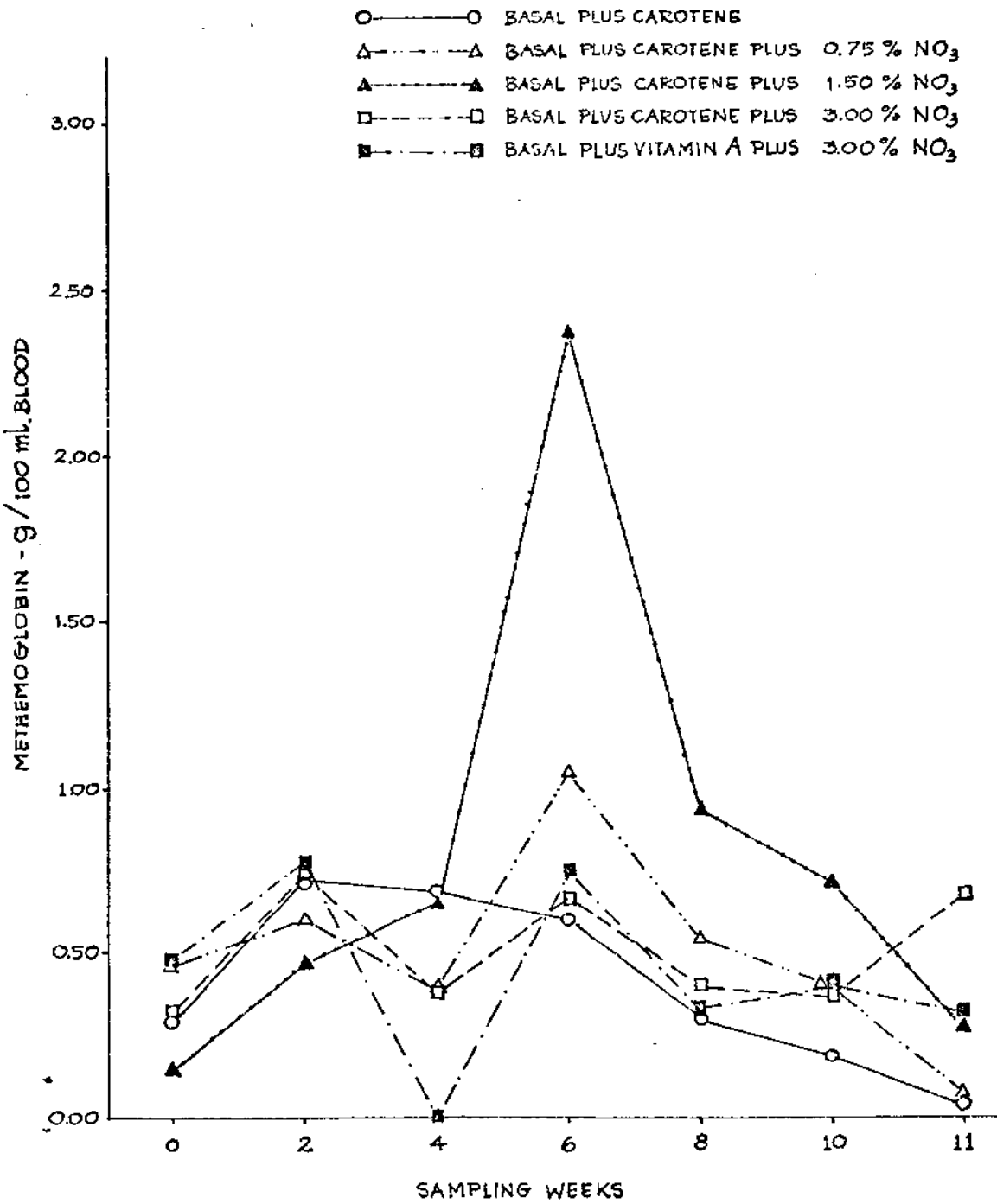


FIG. 6 METHEMOGLOBIN AT 2- WK INTERVALS FOR TRIAL II

Treatment means of the hemoglobin levels were about the same. The range of the final collection period was from 10.3 to 10.7 gram per 100 ml. blood for the pigs on Treatment V and I, respectively. Several other workers data (Koch et al., 1963; Jones et al., 1966) were in agreement with this study. They reported that dietary nitrate showed only a slight difference or had no demonstrable effect on hemoglobin levels.

The average hemoglobin levels for all treatments are plotted in Figure 7. The pigs on Treatment IV and V, had lower final levels (10.4 and 10.3 gram per 100 ml. blood) than the initial levels (11.8 and 10.7 gram per 100 ml. blood). This contrasted with the final levels on Treatment I through III. They have higher levels (10.7, 10.6 and 10.5 gram per 100 ml. blood) compared to the initials (9.98, 9.90 and 9.75 gram per 100 ml. blood).

The average hematocrit values were shown in Figure 8. The final levels for the entire treatments were higher than the initial levels. As the nitrate level increased, the hematocrit level tended to decrease. However, the values are within normal ranges according to the reports of Albritton (1952), Swenson et al. (1958) and Miller et al. (1961). The range was from 38.5 to 42.5 per cent for Treatment I and II, respectively. It can be concluded that the feeding of nitrate did not markedly alter hemoglobin and hematocrit levels.

Trial III

This trial was conducted to study the effects of dietary potassium nitrite (KNO_2) on liver vitamin A depletion, plasma vitamin

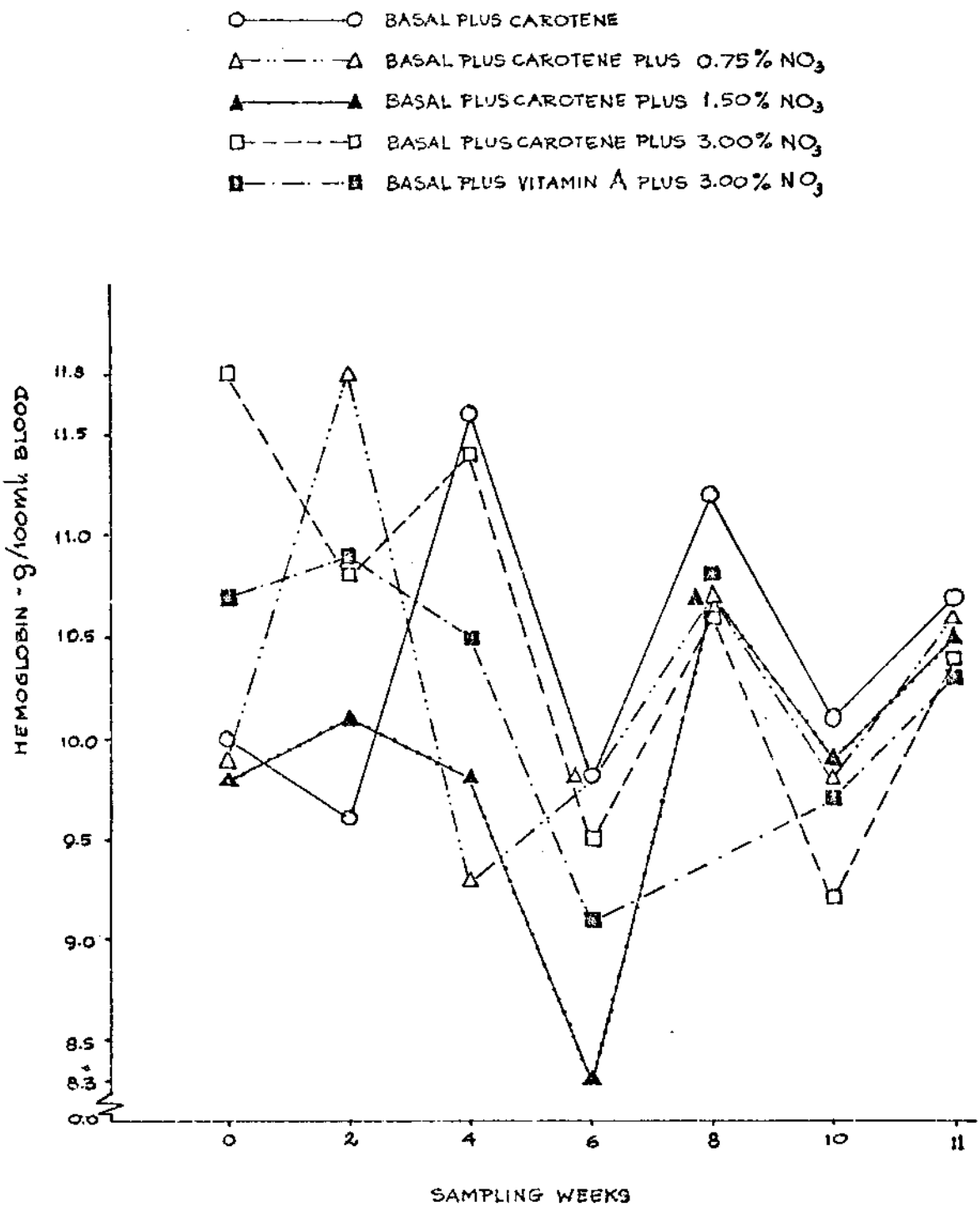


FIG. 7 HEMOGLOBIN FORMATION IN BLOOD OBTAINED FROM PIGS AT 2-WK INTERVALS FOR TRIAL II

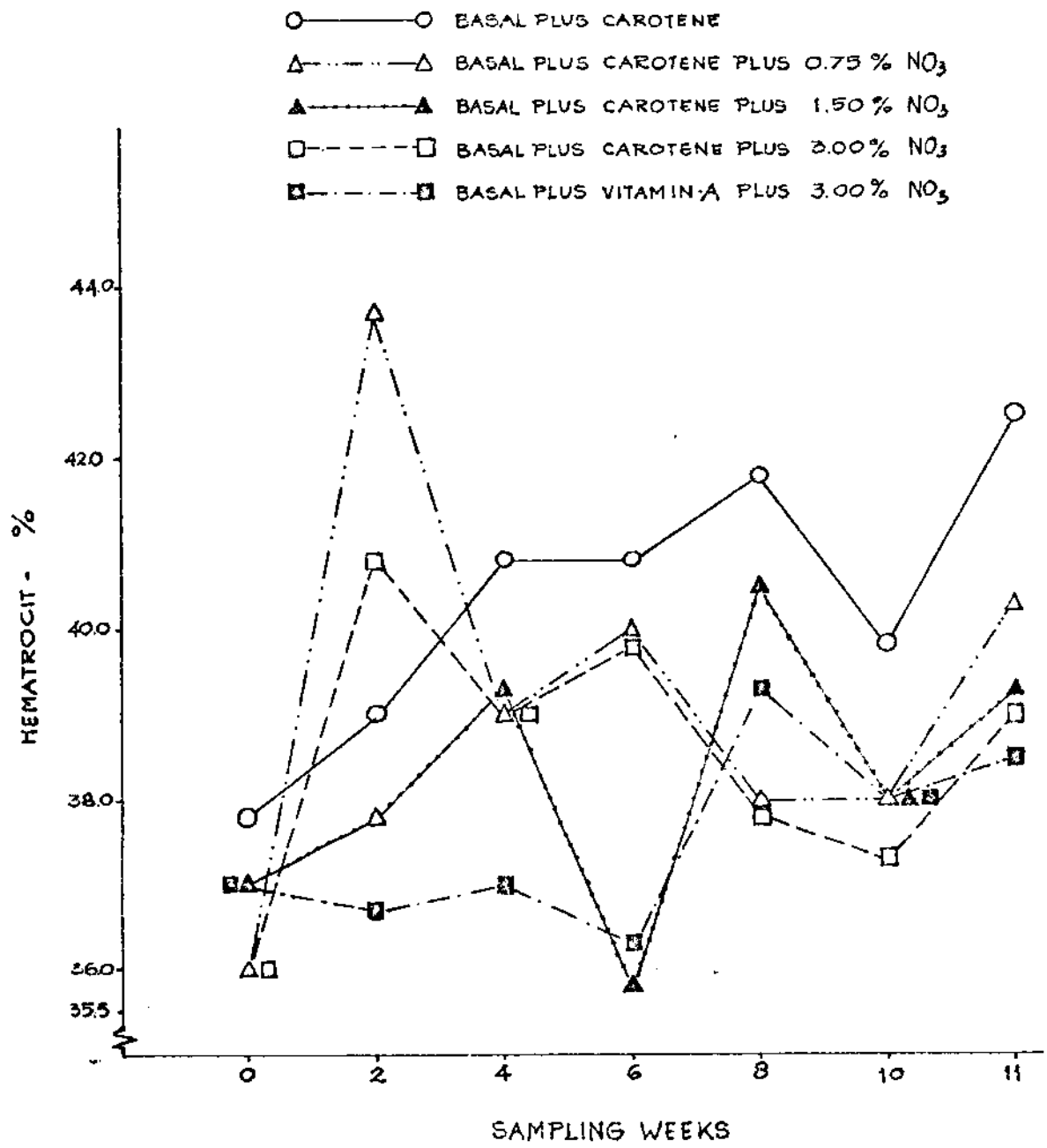


FIG. 8. HEMATROCIT FORMATION IN BLOOD OBTAINED FROM PIGS AT 2-WK INTERVALS FOR TRIAL II

A levels, methemoglobin, hemoglobin and hematocrit blood levels and performance of the pigs.

Throughout the length of the trial the general health of the pigs appeared to be normal. Symptoms were observed on pigs receiving Treatment IV and V as described for Trial I.

A summary of the average daily gain, average amount of feed required per pound of gain and average daily feed consumed, are shown in Table 16. The average daily gains ranged from 1.13 to 1.49 pounds for the pigs receiving Treatments V and I, respectively. The average amount of feed required per pound of gain ranged from 3.65 pounds for Treatment IV to 3.90 pounds for Treatments I and V. No apparent change in feed efficiency was obtained by the feeding of nitrite, which was in contrast with the report of Koch et al. (1963) that the addition of nitrite in the ration depressed feed efficiency of growing pigs.

Analysis of variance test of the average daily gain indicated a significant difference ($P < .01$) between treatments. Treatments II and III were significantly different ($P < .01$) from Treatments IV and V. The average daily gain on 0.3 per cent nitrite-fed pigs receiving vitamin A was not significantly different when compared to 0.3 per cent nitrite-fed pigs receiving carotene. Treatments IV and V were the only ones to show a significant difference ($P < .01$) from the control. This result was supported with the work of Koch et al. (1963) who indicated that the addition of sodium nitrite above 0.3 per cent depressed gain in growing pigs.

TABLE 16

Results of Pig Performance for Trial III

Treatment	Average Initial Weight (Lbs.)	Average Final Weight (Lbs.)	Average Daily Gain (Lbs.)	Feed Per Lb. Gain (Lbs.)	Average Daily Feed Consumption
I (Control)	59.1	196.5	1.49	3.90	5.80
II	56.2	187.9	1.43	3.77	5.38
III	57.6	184.8	1.38	3.73	5.15
IV	57.4	168.7	1.21**	3.65	4.39
V	56.9	160.9	1.13**	3.90	4.40

**$P < .01$

Treatment means of the plasma vitamin A levels for the initial, mid-trial and final collection is summarized in Table 17. The range of the final collection was from 11.7 to 20.3 mcg. of vitamin A per 100 ml. blood for Treatment V and I, respectively.

Analysis of variance test of the final plasma vitamin A levels indicated a significant difference ($P < .05$) between treatments. Plasma vitamin A levels on low nitrite - fed pigs (Treatment II and III) were significantly different ($P < .05$) when compared to high nitrite - fed pigs (Treatment IV and V). The feeding of a nitrite - vitamin A ration (Treatment V) did not show a significant difference in plasma vitamin A level compared to a nitrite - carotene ration (Treatment IV). But, it tended to be lower on Treatment V. Comparing the control with the other treatment means, treatment V was the only one found to be significantly different ($P < .05$). This was in agreement with the work of Koch et al. (1963) who reported that the addition of 0.3 to 0.5 per cent of sodium nitrite reduced the plasma vitamin A level.

An attempt was precluded to obtain a relationship between nitrite level and plasma vitamin A by using linear regression test (Snedecor, 1965; Steel and Torrie, 1960; Ezekiel and Fox, 1963). Treatment levels were used as independent variables with plasma vitamin A levels as dependent variables. There was a negative relationship ($P < .01$) between the levels of nitrite and plasma vitamin A. As the nitrite increased, plasma vitamin A level decreased with b (sample regression coefficient) = - 12.66 mcg. per 100 ml. blood.

TABLE 17

Results of Average Plasma Vitamin A Levels

(mcg. per 100 ml. blood) for Trial III

Treatment	Initial Collection (1st day)	Mid-trial Collection (42nd day)	Final Collection (87th day)
I	28.7 (2.62)[a]	17.9 (1.74)	20.3 (2.40)
II	31.2 (6.56)	16.0 (6.39)	19.7 (5.91)
III	30.9 (9.04)	15.1 (3.97)	17.3 (2.43)
IV	38.8 (9.71)	12.8 (1.89)	16.0 (2.38)
V	33.3 (3.44)	15.1 (4.26)	11.7* (0.42)

[a]Standard deviations

*$P < .05$

The plasma vitamin A values are plotted in Figure 9. There was a decline for all treatments during the first to the sixth (mid-trial) week, with the exception of Treatment V that continued to decline to the end of the trial. Starting from the sixth week, plasma vitamin A formation on Treatments I through IV showed no significant changes to the termination of the trial. Plasma vitamin A levels for all treatments were lower than the initial levels. The lower levels were found in pigs receiving Treatment IV and V, which had the higher nitrite levels.

Standard liver vitamin A levels for this trial 67.84 mcg. per gram of liver tissue were as described for Trial II. The average final liver vitamin A stores ranged from 9.48 to 21.13 mcg. per gram of liver tissue for the pigs on Treatment V and I, respectively.

Analysis of variance test of the average liver storage of vitamin A indicated no significant differences between control and other treatments. In contrast, Emerick and Olson (1962) showed in rats that the feeding of 0.5 per cent sodium nitrite reduced liver vitamin A stores when vitamin A or carotene was administered orally. Also O'Dell et al. (1960) reported that a diet containing 0.3 per cent of sodium nitrite reduced liver vitamin A level in rats.

Treatment means for the methemoglobin, hemoglobin and hematocrit levels are presented in Table 18. Analysis of variance tests of the average final blood methemoglobin levels indicated a significant differences ($P < .01$) between control and other treatments. The pigs on high nitrite levels (Treatments IV and V) significantly

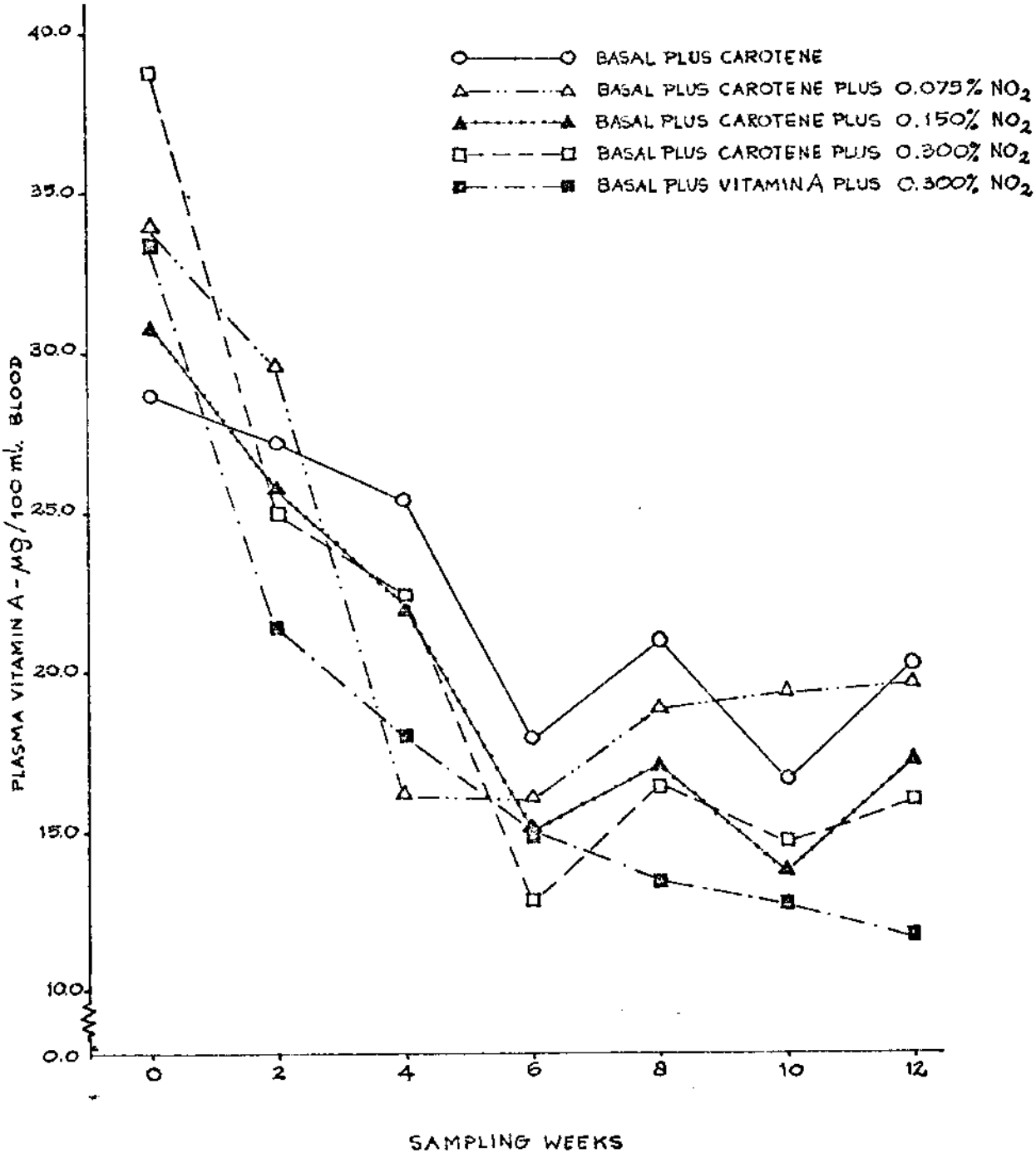


FIG. 9 PLASMA VITAMIN A AT 2-WK INTERVALS FOR TRIAL III

TABLE 18

Results of Average Methemoglobin, Hemoglobin, and Hematocrit for Trial III

Treatment	Sampling Weeks						
	0	2	4	6	8	10	12
I							
Methemoglobin[a]	0.36	0.75	0.42	0.18	0.33	0.47	0.20
Hemoglobin[a]	9.60	11.03	9.23	9.85	9.70	9.15	9.98
Hematocrit[b]	37.33	41.25	35.75	40.00	38.33	37.75	39.80
II							
Methemoglobin	0.19	1.22	0.32	0.86	0.33	0.28	0.20
Hemoglobin	9.40	9.88	9.97	8.80	9.67	9.75	10.43
Hematocrit	34.33	40.00	39.75	38.67	36.00	37.50	39.00
III							
Methemoglobin	0.23	3.48	2.13	2.05	0.70	1.44	0.25
Hemoglobin	9.90	8.15	7.30	7.85	9.98	7.93	9.53
Hematocrit	39.25	34.50	33.00	36.00	34.75	34.00	37.75
IV							
Methemoglobin	0.38	6.70	3.94	5.39	2.31	2.56	1.44
Hemoglobin	10.28	8.33	8.38	6.45	9.23	9.30	8.85
Hematocrit	37.50	33.75	34.75	33.00	36.50	36.25	36.75
V							
Methemoglobin	0.17	4.26	5.59	4.74	2.66	1.83	1.35
Hemoglobin	10.10	9.60	8.58	8.40	7.93	7.28	8.03
Hematocrit	39.33	37.67	34.75	37.50	34.33	33.00	34.50

[a]Methemoglobin and Hematocrit measured in gram per 100 ml. blood.

[b]Hematocrit measured in per cent of blood.

increased ($P < .01$) methemoglobin levels compared to low nitrite levels (Treatment II and III). Blood methemoglobin levels on nitrite-fed pigs receiving carotene (Treatment IV) were significantly different from nitrite-fed pigs receiving vitamin A (Treatment V). Treatments IV and V were the only ones to reveal a significant difference ($P < .01$) from the control. Similar observations were made by Koch et al. (1963) who reported that the addition of 0.3 to 0.5 per cent sodium nitrite increased methemoglobin levels.

The results of the average methemoglobin levels for the initial, mid-trial and final collection periods are summarized in Table 19. The range of the final collection was from 0.20 to 1.44 gram per 100 ml. blood for Treatments I and IV, respectively.

Methemoglobin values for all treatments are plotted in Figure 10. The highest peak was found during the second week for Treatment IV, 6.70 gram per 100 ml. blood. It was followed by the peaks for Treatment V during the fourth week, 5.59 gram per 100 ml. blood and during the sixth week on Treatment IV, 5.39 gram per 100 ml. blood. Methemoglobin level for Treatment I and II remained consistent throughout the length of the trial. Treatment III had intermediate levels compared to the other treatments. Its highest peak, 3.48 gram per 100 ml. blood was observed during the second week of the trial. This indicated that the feeding of nitrite enhanced the maximum formation during the second to the sixth week of the trial. The final methemoglobin formation remained high on Treatment IV and V in comparison with the other treatments.

TABLE 19

Results of Average Methemoglobin Levels

(gram per 100 ml. blood) for Trial III

Treatment	Initial Collection (1st day)	Mid-trial Collection (42nd day)	Final Collection (87th day)
I	0.36 (0.20)[a]	0.18 (0.15)	0.20 (0.16)
II	0.19 (0.32)	0.86 (0.48)	0.20 (0.16)
III	0.23 (0.28)	2.05 (1.90)	0.25 (0.25)
IV	0.38 (0.34)	5.39 (2.17)	1.44 (0.64)
V	0.17 (0.18)	4.74 (1.11)	1.35 (0.38)

[a]Standard deviations

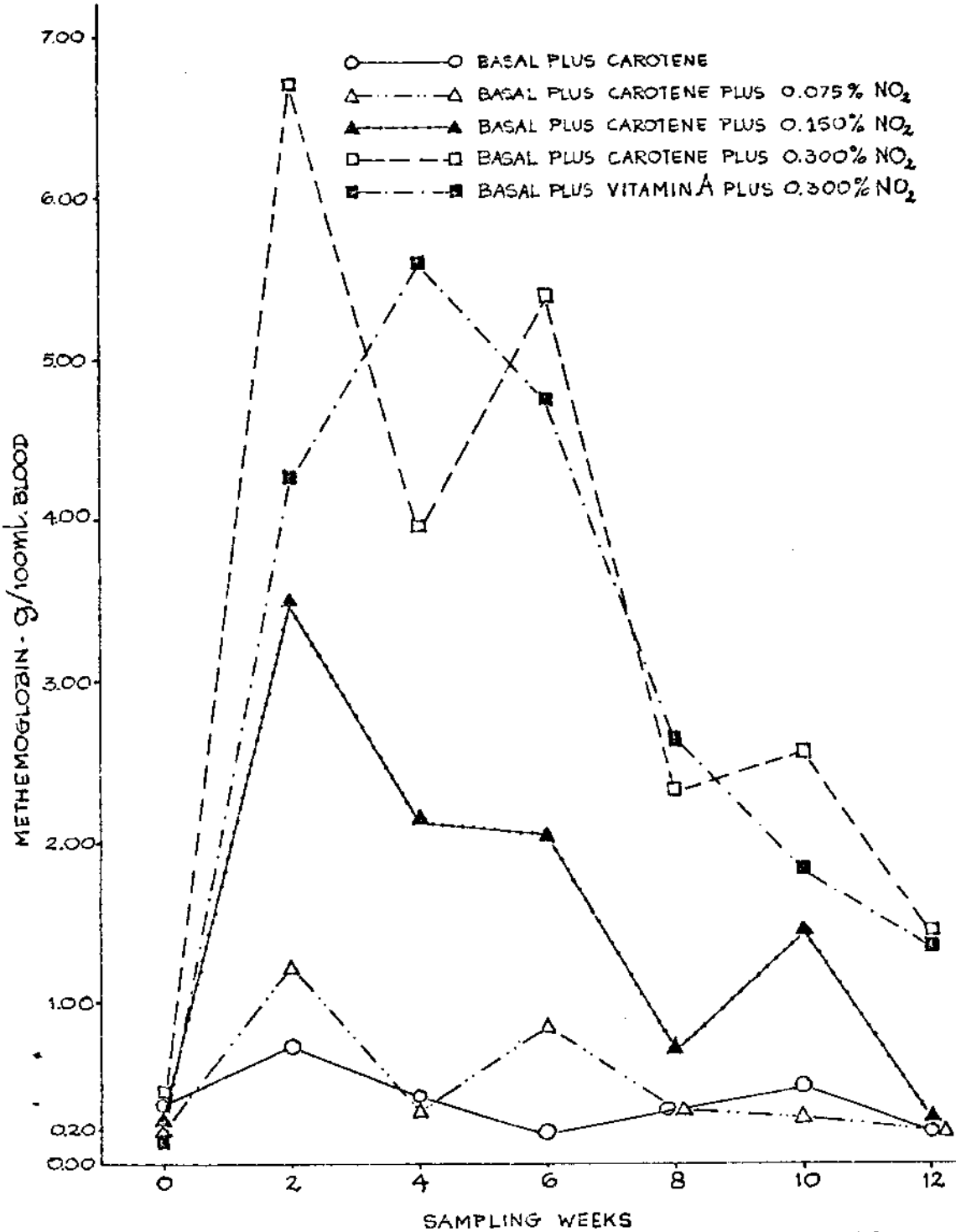


FIG. 10 METHEMOGLOBIN AT 2-WK INTERVALS FOR TRIAL III

Linear regression tests indicated positive relationship ($P < .01$) between nitrite and methemoglobin level. As the nitrite level increased, methemoglobin level increased with b = 2.46 gram per 100 ml. blood.

The average hemoglobin levels for all treatments are shown in Figure 11. They remained consistent during the entire trial period. This is in agreement with the report of Koch et al. (1963) that dietary nitrite had no demonstrable effect on hemoglobin levels. Treatment IV and V were found to have a lower final level, 8.85 and 8.03 gram per 100 ml., respectively compared to the other treatments.

The average hematocrit values are plotted in Figure 12. The values were similar although there was a tendency to be lower as the nitrite level in the diet increased.

It was presumed that hemoglobin and hematocrit levels tended to be lower on nitrite-vitamin A ration than on nitrite-carotene ration. However, they were within the ranges reported by Albritton (1952), Swenson et al. (1958) and Miller et al. (1961).

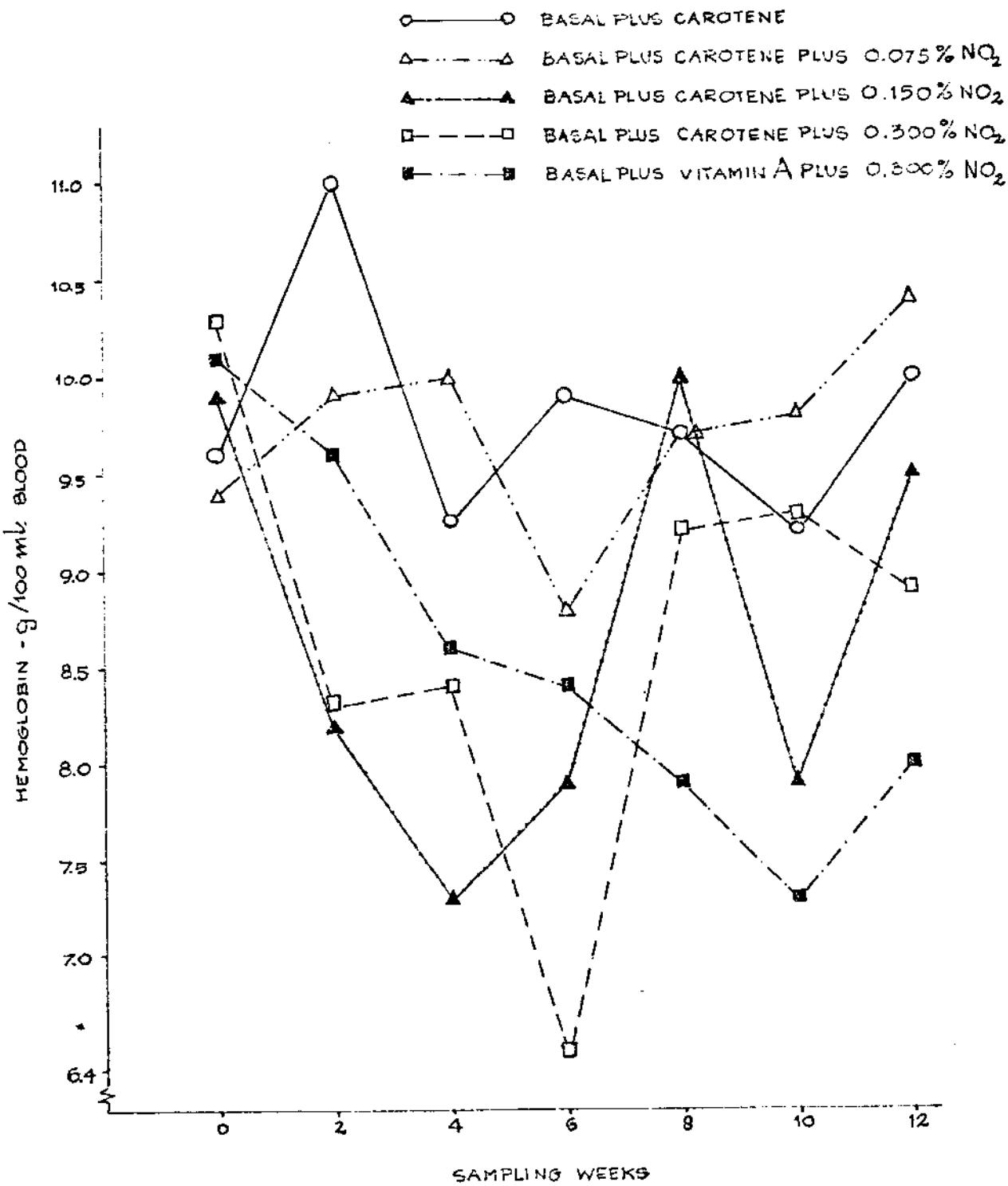


FIG. 11 HEMOGLOBIN FORMATION IN BLOOD OBTAINED FROM PIGS AT 2-WK INTERVALS FOR TRIAL III

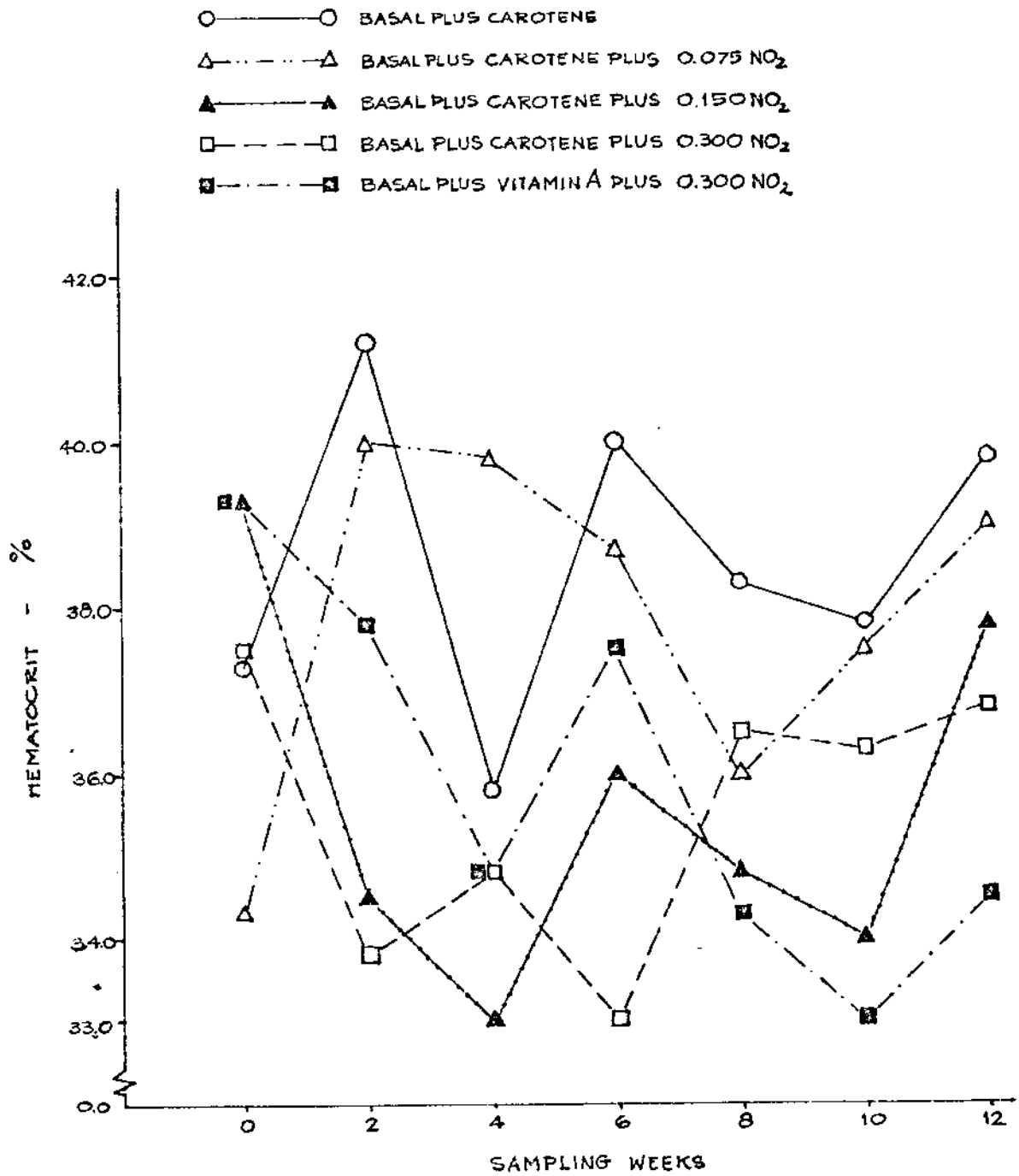


FIG. 12 HEMATOCRIT FORMATION IN BLOOD OBTAINED FROM PIGS AT 2-WK INTERVALS FOR TRIAL III

CHAPTER V

SUMMARY

Trial I

Fifty Yorkshire x Hampshire cross pigs averaging 56 pounds were allotted to ten lots according to sex and weight, to study the effects of potassium nitrate and potassium nitrite in the diet on the depletion of liver vitamin A stores, plasma vitamin A, methemoglobin, hemoglobin, hematocrit levels and performance. Supplementary vitamin A was added to the ration until the pigs averaged 100 poundds to ensure adequate vitamin A stores in the liver. Two lots of five pigs were randomly assigned to each of the following ration treatments: (1) 0% NO_3 and NO_2, (2) 0.075 % NO_2, (3) 0.300 % NO_2, (4) 0.750% NO_3 and (5) 3.00% NO_3. The pigs were fed a balanced ration with the exception of vitamin A or carotene. Liver samples collected at the beginning and at the end of the trial, were analyzed for vitamin A stores. Blood samples were obtained bi-weekly and analyzed for plasma vitamin A, methemoglobin, hemoglobin, and hematocrit levels.

Average daily gains were significantly reduced ($P < .01$) by the feeding of 3 per cent nitrate or 0.3 per cent nitrite. Feed efficiency tended to decrease as the per cent of nitrate in feed increased.

The addition of nitrate and nitrite to the diet did not significantly affect plasma vitamin A. Although, there was a trend for a decrease as the level of nitrate and nitrite increased.

Liver vitamin A stores were not markedly influenced either by dietary nitrate or nitrite. However, liver vitamin A stores tended to decrease as the level of nitrate and nitrite in the diet increased.

No differences in the rate of methemoglobin formation were apparent by either nitrate or nitrite. But, it could be presumed that dietary nitrate or nitrite tended to enhance methemoglobin formation.

Hemoglobin and hematocrit readings remained normal throughout on the length of the trial.

Trial II

Six purebred Hampshire and thirty-four purebred Yorkshire pigs of an average initial weight of 57 pounds were allotted to ten lots according to breed, sex and weight, to study the effects of potassium nitrate on the depletion of liver vitamin A stores, plasma vitamin A, methemoglobin, hemoglobin, hematocrit and performance. Two lots of four pigs each were randomly assigned to each of the following treatments: (1) 0% NO_3, (2) 0.75% NO_3, (3) 1.50% NO_3, (4) 3.00% NO_3 and (5) 3.00% NO_3. Treatment I through IV received supplemental carotene equivalent to 500 I.U. of true vitamin A per pound of feed. Treatment V obtained 500 I.U. of true vitamin A per pound of feed. Liver samples collected at the beginning and at the end of the trial were analyzed for vitamin A stores. Blood samples collected bi-weekly were analyzed for plasma vitamin A, methemoglobin and hematocrit levels.

The addition of 3 per cent nitrate to ration containing supplemental carotene or vitamin A significantly reduced gains ($P < .01$). No differences were found between the nitrite-vitamin A ration (Treatment V) and the nitrate-carotene ration (Treatment IV) on the average daily gain. Feed efficiency tended to decrease as the nitrate level in the feed increased.

Dietary nitrate did not significantly influence plasma vitamin A. But, there was a tendency to reduce plasma vitamin A as the nitrate level in the diet increased.

The feeding of nitrate had no demonstrable effect on liver storage of vitamin A. However, there was a tendency for a decrease as the level of nitrate in the diet increased.

Dietary nitrate did not significantly increase methemoglobin formation. Although it tended to increase as the nitrate level in the diet increased. Hemoglobin and hematocrit formations remained relatively consistent throughout the length of the trial.

Trial III

Seven purebred Hampshire and thirty-three purebred Yorkshire pigs averaging 58 pounds were allotted to ten lots according to breed, sex and weight, to study the effects of potassium nitrite on the depletion of liver vitamin A stores, plasma vitamin A, methemoglobin, hemoglobin, hematocrit and performance. Two lots of four pigs each were randomly assigned to each of the following treatments: (1) 0% NO_2,

(2) 0.075% NO_2, (3) 0.150% NO_2, (4) 0.300% NO_2 and (5) 0.300% NO_2. Treatment I through IV received supplemental carotene equivalent to 500 I.U. of true vitamin A per pound of feed. Treatment V received 500 I.U. of vitamin A per pound of feed. Liver samples obtained at the beginning and at the end of the trial, were analyzed for vitamin A stores. Blood samples collected bi-weekly were analyzed for plasma vitamin A, methemoglobin and hematocrit levels.

Average daily gains were significantly depressed ($P < .01$) by the addition of 0.3 per cent nitrite regardless of whether the pigs received carotene or vitamin A. High nitrite-fed pigs (0.3% NO_2) reduced gains significantly ($P < .01$) when compared to low-nitrite-fed pigs (0.075% and 0.150% NO_2). No differences in effect of nitrite-vitamin A ration (Treatment V) on the average daily gains was noted when compared with nitrite-carotene ration (Treatment IV). However, the average daily gains tended to be lower on the nitrite-vitamin A ration. Feed efficiency tended to decrease as the level of nitrite in the diet increased, with the exception of the nitrite-vitamin A ration (Treatment V).

The feeding of 0.3 per cent nitrite where carotene or vitamin A was supplemented significantly reduced plasma vitamin A values ($P < .05$). Plasma vitamin A level in high nitrite-fed pigs (Treatment IV and V) was significantly lower ($P < .05$) than in low nitrite-fed pigs (Treatments II and III). Pigs on the nitrite-vitamin A ration (Treatment V) tended to have lower plasma vitamin A levels than those on the nitrite-carotene ration (Treatment IV), although it did not show a significant effect.

Dietary nitrite markedly influenced plasma vitamin A levels. This indicates that plasma vitamin A decreased as the nitrite level increased.

The liver vitamin A stores was not reduced by dietary nitrite. However, they tended to decrease as the nitrite level increased.

The feeding of 0.3 per cent nitrite (Treatments IV and V) significantly ($P < .01$) increased methemoglobin values. High nitrite-fed pigs exhibited a faster rate ($P < .01$) of methemoglobin formation compared to low nitrite-fed pigs. Neither nitrite - carotene nor nitrite - vitamin A rations revealed a significant difference in the rate of methemoglobin formation.

Hematocrit and hemoglobin readings remained consistent during the entire trial period.

CHAPTER VI

CONCLUSIONS

Under conditions of this experiment, the following conclusions seem justified:

1. The feeding of nitrate and nitrite significantly depresses gain of growing pigs fed supplemental carotene or vitamin A.
2. The feeding of nitrate and nitrite tends to depress feed utilization.
3. The feeding of nitrite, but not nitrate, to pigs receiving supplemental carotene or vitamin A reduces plasma vitamin A levels.
4. The feeding of nitrate and nitrite tends to reduce liver vitamin A stores of pigs.
5. The feeding of nitrate and nitrite tends to increase methemoglobin synthesis.
6. The feeding of nitrate or nitrite to growing pigs receiving supplemental vitamin A tends to show a faster depletion rate of liver vitamin A stores and reduction of plasma vitamin A when compared to nitrite-fed pigs receiving supplemental carotene.
7. Dietary nitrite significantly affects plasma vitamin A and methemoglobin levels during the first to the sixth week of feeding when either carotene or vitamin A palmitate is the source of vitamin A activity.

APPENDIX

APPENDIX TABLE 1

Analysis of Variance of Average Daily Gain for Trial I

Source of Variation	d.f.	S.S.	M.S.	F
Replication	1	0.0036	0.0036	0.21
Treatments	4	0.9218	0.2304	13.24**
Error	34	0.5918	0.0174	
Total	39	1.5172		

**Significant difference ($P < .01$)

APPENDIX TABLE 2

Analysis of Variance of Average Daily Gain for Trial II

Source of Variation	d.f.	S.S.	M.S.	F
Season	1	0.5018	0.5018	11.03**
Treatments	4	1.0308	0.2577	5.67**
Treatments vs. Season	4	0.09	0.0225	0.49
Error	28	1.2742	0.0455	
Total	37	2.8968		

**Significant difference ($P < .01$)

APPENDIX TABLE 3

Analysis of Variance of Average Daily Gain for Trial III

Source of Variation	d.f.	S.S.	M.S.	F
Season	1	0.1232	0.1232	3.35
Treatments	4	0.737	0.1842	5.00**
Treatments vs. Season	4	0.018	0.0045	0.12
Error	29	1.0682	0.0368	
Total	38	1.9464		

**Significant difference ($P < .01$)

APPENDIX TABLE 4

Analysis of Variance of Liver Vitamin A for Trial I

Source of Variation	d.f.	S.S.	M.S.	F
Treatments	4	159.0258	39.7564	2.27
Error	15	262.9048	17.5269	
Total	19	421.9306		

APPENDIX TABLE 5

Analysis of Variance of Liver Vitamin A
for Trial II

Source of Variation	d.f.	S.S.	M.S.	F
Treatments	4	139.2284	34.8071	0.38
Error	13	1200.5551	92.3503	
Total	17	1339.7835		

APPENDIX TABLE 6

Analysis of Variance of Liver Vitamin A
for Trial III

Source of Variation	d.f.	S.S.	M.S.	F
Treatments	4	368.6680	92.1670	2.60
Error	15	530.8301	35.3886	
Total	19	899.4981		

APPENDIX TABLE 7

Analysis of Variance of Plasma Vitamin A
for Trial I

Source of Variation	d.f.	S.S.	M.S.	F
Treatments	4	123.433	30.86	2.62
Error	15	176.720	11.78	
Total	19	300.150		

APPENDIX TABLE 8

Analysis of Variance of Plasma Vitamin A
for Trial II

Source of Variation	d.f.	S.S.	M.S.	F
Treatments	4	82.5686	20.6421	0.54
Error	12	459.0809	38.2567	
Total	16	541.6495		

APPENDIX TABLE 9

Analysis of Variance of Plasma Vitamin A
for Trial III

Source of Variation	d.f.	S.S.	M.S.	F
Treatments	4	190.3720	47.5930	4.74*
Error	15	150.5775	10.0385	
Total	19	340.9495		

*Significance difference ($P < .05$)

APPENDIX TABLE 10

Analysis of Variance of Methemoglobin
for Trial I

Source of Variation	d.f.	S.S.	M.S.	F
Treatments	4	6.7313	1.6828	2.30
Error	15	10.9540	0.7302	
Total	19	17.6853		

APPENDIX TABLE 11

Analysis of Variance of Methemoglobin
for Trial II

Source of Variation	d.f.	S.S.	M.S.	F
Treatments	4	1.0197	0.2549	1.20
Error	13	2.7608	0.2123	
Total	17	3.7805		

APPENDIX TABLE 12

Analysis of Variance of Methemoglobin
for Trial III

Source of Variation	d.f.	S.S.	M.S.	F
Treatments	4	6.6876	1.6719	12.64**
Error	15	1.9848	0.1323	
Total	19	8.6724		

**Significance difference ($P < .01$)

APPENDIX TABLE 13

Results of Average Hemoglobin and Hematocrit Levels for Trial I

Treatment	Initial Collection (1st day)	Mid-trial Collection (28th day)	Final Collection (56th day)
I			
Hemoglobin[a]	10.95	11.58	11.90
	(0.17)[c]	(0.60)	(2.24)
Hematocrit[b]	40.25	40.50	44.33
	(2.03)	(1.73)	(2.33)
II			
Hemoglobin	11.83	11.25	11.53
	(0.85)	(0.39)	(0.85)
Hematocrit	42.50	40.50	43.50
	(1.29)	(1.29)	(1.29)
III			
Hemoglobin	10.95	10.05	10.13
	(0.62)	(1.50)	(0.91)
Hematocrit	40.00	37.50	35.33
	(2.94)	(4.93)	(3.79)
IV			
Hemoglobin	11.00	12.00	11.13
	(1.21)	(0.57)	(1.84)
Hematocrit	40.00	43.25	43.50
	(2.83)	(1.26)	(2.52)
V			
Hemoglobin	10.95	11.58	11.25
	(0.62)	(0.57)	(0.30)
Hematocrit	41.00	42.00	42.25
	(2.16)	(1.16)	(3.05)

[a]Hemoglobin measured in gram per 100 ml. of blood

[b]Hematocrit measured in per cent of blood

[c]Standard deviations

APPENDIX TABLE 14

Results of Average Hemoglobin and Hematocrit Levels for Trial II

Treatment	Initial Collection (1st day)	Mid-trial Collection (42nd day)	Final Collection (80th day)
I			
Hemoglobin[a]	9.98	9.83	10.73
	(1.17)[c]	(0.37)	(0.28)
Hematocrit[b]	37.75	40.75	42.50
	(1.26)	(2.10)	(1.92)
II			
Hemoglobin	9.90	9.80	10.60
	(0.42)	(1.25)	(1.00)
Hematocrit	36.00	40.00	40.33
	(0.00)	(4.58)	(2.52)
III			
Hemoglobin	9.75	8.30	10.50
	(1.65)	(1.26)	(0.55)
Hematocrit	37.00	35.75	39.25
	(1.00)	(4.03)	(1.46)
IV			
Hemoglobin	11.83	9.45	10.42
	(0.67)	(0.52)	(0.99)
Hematocrit	36.00	39.75	39.00
	(1.00)	(0.96)	(1.41)
V			
Hemoglobin	10.70	9.10	10.30
	(0.62)	(0.62)	(0.00)
Hematocrit	37.00	36.33	38.50
	(3.00)	(1.53)	(0.71)

[a]Hemoglobin measured in gram per 100 ml. of blood

[b]Hematocrit measured in per cent of blood

[c]Standard deviations

APPENDIX TABLE 15

Results of Average Hemoglobin and Hematocrit Levels for Trial III

Treatment	Initial Collection (1st day)	Mid-trial Collection (42nd day)	Final Collection (87th day)
I			
Hemoglobin[a]	9.60	9.85	9.98
	(1.31)[c]	(1.26)	(0.89)
Hematocrit[b]	37.33	40.00	39.80
	(0.58)	(3.37)	(2.22)
II			
Hemoglobin	9.40	8.80	10.43
	(1.14)	(0.92)	(1.08)
Hematocrit	34.33	38.67	39.00
	(3.51)	(2.08)	(1.15)
III			
Hemoglobin	9.90	7.85	9.53
	(1.41)	(1.54)	(1.35)
Hematocrit	39.25	36.00	37.75
	(1.09)	(6.38)	(3.40)
IV			
Hemoglobin	10.28	6.45	8.85
	(0.62)	(0.64)	(1.11)
Hematocrit	37.50	33.00	36.75
	(2.38)	(2.71)	(2.22)
V			
Hemoglobin	10.10	8.40	8.03
	(0.46)	(0.42)	(1.68)
Hematocrit	39.33	37.50	34.50
	(2.08)	(0.57)	(3.90)

[a]Hemoglobin measured in gram per 100 ml. of blood

[b]Hematocrit measured in per cent of blood

[c]Standard deviations

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BIOGRAPHICAL SKETCH

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