# SOME EFFECTS OF DIETARY NITRATE AND NITRITE ON METHEMOGLOBIN LEVEL, CAROTENE CONVERSION, WEIGHT GAIN AND FEED EFFICIENCY IN GROWING SWINE

by

SIRILPONG SUKHONTHASARNPA

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Earl A. Koch

Major Professor

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# INTRODUCTION

This thesis reports a study of the effects of nitrate and nitrite, the sodium salts, upon growing swine when fed at sublethal doses. Intake of such levels can apparently occur anywhere on the earth. The literature cited herein contains many reports indicating that farm animals suffered from nitrate and nitrite poisoning due to the ingestion of plant materials that contained high nitrate or drinking water that contained high nitrate or nitrite, or both. Case (1963) presented a review designating places in the United States where nitrate and nitrite were present in well water. Northeast Kansas was one of the areas so designated. Reports of nitrate and nitrite poisoning in Africa, Australia, Europe and South America have appeared in various scientific journals.

The purposes of this study were to specifically study the effects of various levels of nitrate and nitrite intake on carotene and vitamin A metabolism, effects on hemoglobin and methemoglobin levels and the effects upon general performance of growing swine. The experiments were carried out by feeding swine rations supplemented with various levels of sodium nitrate and sodium nitrite to produce the chronic and sublethal effects in order to obtain the information desired.

## REVIEW OF LITERATURE

There are numerous reports and reviews concerned with the causes, symptoms and treatments of nitrate and nitrite poisoning in different classes of livestock. As reviewed by Whitehead and Maxon (1952), well water and plant materials containing high nitrate and nitrite levels appear to be the usual cause for nitrate and nitrite toxicity symptoms. Deficiency of certain trace minerals (Cu, Co, Mn), level of soil nitrate content, stage of plant growth, application of herbicides, shortage of sunlight for proper photosynthesis, drought and certain plant species' characteristics may all be important causes of increased accumulation of nitrate and nitrite in plant materials.

McIntosh and Wilson (1943) reported that the feeding of boiled mangold to 20 kg. pigs, so that each pig obtained the equivalent of approximately 1-3 gm. of $NaNO_2$ per day, gave symptoms of toxicity but the pigs did not die. They also reported one case where cooked mangold caused death in pigs within one-half hour after feeding; two hundred out of six hundred head in the herd died after showing such symptoms as labored respiration, pain and muscular weakness.

Wink, Sutherland and Salisbury (1950) observed a heavy mortality in pigs fed on soup prepared by cooking beef offals in well water containing 1740-2970 ppm of $NaNO_2$ equivalent. The analyses of two batches of soup showed that they contained 393 and 1127 ppm of $NaNO_2$ equivalent. In a toxicity experiment they found that feeding 0.05-0.08 gm. of $NaNO_2$ per kg. body weight produced moderate to severe non-fatal methemoglobinuria. Two pigs, weighing 61 and 80 kg., died two hours after an oral dose of

0.09 gm. per kg. body weight. The pigs were fasted 18 hours before treatment.

Gwatkin and Plummer (1946) reported that 30-90 gm. of $NaNO_3$ and 5 gm. of $NaNO_2$ were deadly doses for small fasting pigs. Small pigs were fatally poisoned by oral doses of 5 gm. $NaNO_2$ when fasting but were not markedly affected when the stomach was full.

Hvidsten (1955), fed pigs as much as 4 mg. of $NaNO_2$ per kg. body weight per day to study the toxicity of a herring meal prepared from herring preserved with $NaNO_2$. He reported that the levels of $NO_2^-$ fed had no effect on growth rate, feed efficiency or iodine number of the fat. Nitrite feeding at the levels he used had no effect on vigor and there were no visible signs of any effect upon health, but methemoglobin increased to 9% of total hemoglobin. Nitrite in the feed reduced the amount of vitamin A deposited in the liver. In one experiment it was observed that when both cod-liver oil and nitrite were present in feed there was practically no deposition of liver vitamin A.

Kilgore, Almon and Gregor (1959) reported increased levels of methemoglobin in the blood of rabbits and rats within 7-14 hours after a meal giving 0.2, 0.4 or 1.0 gm. of $NaNO_3$ per kg. body weight. High methemoglobin values were also found in rabbits after feeding collards containing 0.61% of $NaNO_3$ equivalent in total dry matter and in rats after feeding turnip greens which contained 8.85% of $NaNO_3$ in the total dry matter. No signs of discomfort or cyanosis were evident in any of the animals. In mineral-balance studies on rabbits, it was found that only 35.3 to 56.1% of the $NO_3^-$ given was recovered in the urine within a 24-hour period. Small amounts of nitrite were also found in urine.

Holtenius (1957), reported an increase of both $O_2$ consumption and $CO_2$ output following the intravenous injection of 5 to 35 mg. of $NaNO_2$ per kg. body weight into sheep. He suggested that the increases resulted from increased muscular activity. Up to 65% of total hemoglobin could be converted into methemoglobin and the animal could be lying in a coma without any decrease in oxygen consumption. He also reported that procaine penicillin speeded the breakdown of $NO_2^-$. Bradley, Epson and Beath (1940) reported that methylene blue could be used as an antidote for nitrate poisoning resulting from injection of oat's hay containing a high nitrate concentration.

Njaa and Braekkan (1955) fed rats a good mixed diet supplemented with $NaNO_2$ so that the rats received 0.5, 1.0 and 2.0 mg. of $NaNO_2$ per day for 12 weeks. They found no ill-effects, growth was normal, and no abnormal post-mortem lesions were observed even though on one of the treatments total intake of $NaNO_2$ within a period of 70 days was from 70 to 105 mg.

Matsumoto, Hylin and Miyahara (1961) reported that rats survived after the injection of $NaNO_2$ even though methemoglobin levels reached 50% of the total hemoglobin whereas the injection of 3-nitro-propanoic acid caused death when methemoglobin levels were approximately 25%. Incubation of nitroethane or 3-nitroethane or 3-nitro-propanoic acid with blood in vitro did not produce methemoglobin.

Pollard and Bieri (1958), reported that 1/3 to 1/2 of the vitamin A activity of a vitamin A solution (dissolved in tween 40) was destroyed within five minutes after intravenous injection into rats. Destruction was greatest in rats less than 40 days of age. The destructive activity diminished rapidly in older rats. Destruction was less when the solution

was injected into mice and guinea pigs and there was no destruction when the solution was injected into chicks. Incubation of vitamin A with homogenates from different tissues showed that only hemolysed red blood cells destroyed significant amounts of vitamin A. Dmitrovskij (1961) also reported that the oxidation of vitamin A in vitro was effectively catalysed by hematin compounds, especially by acid hematin at pH 4. Methemoglobin and oxyhemoglobin were somewhat less effective, cytochrome c, $Fe^{++}$, $Cu^{++}$, $Fe^{+++}$, $Co^{++}$ were still less effective. Oxidation of vitamin A by carp intestine was more rapid than in a homogenate of ox liver because the former contained a higher level of hemoglobin. With either tissue, oxidation was accelerated by acidification. The presence of fat was necessary for the catalysis of hematin. He also injected rats subcutaneously with a solution of $NaNO_2$ at the rate of 60 mg. per kg. body weight along with orally administered vitamin A and found that as the methemoglobin level of the blood increased the amount of vitamin A stored in the liver decreased. He concluded that the presence of methemoglobin in the body increased the destruction of vitamin A.

McGillivray (1960) upon revieswing his earlier studies proposed that the rate of conversion of carotene given intravenously in aqueous disperaion to vitamin A was increased by incorporation with unsaturated lipids. It was suggested that the initial reaction was coupled with unsaturated lipid oxidation and catalysed by hemoglobin, or when carotene was given by mouth he proposed that the reaction was catalysed by myoglobin in the tissues. Tocopherols inhibited the conversion because of their antioxidant properties.

Metcalf (1962) reported that young and pregnant rats showed sensitivity to oxidation of their intracorpuscular hemoglobin by $NO_2^-$ just as did children and pregnant women. The change of sensitivity did not appear to be correlated with puberty. The effects of starvation were the same as those of pregnancy and extreme youth and the resistance of hemoglobin to $NO_2^-$ could be restored by the administration of riboflavin. Subcutaneous injection of riboflavin increased the resistance of hemoglobin of young and pregnant rats to oxidation by $NO_2^-$.

Garner et al. (1958) fed sows as much as 2.0% of $KNO_3$ in the total ration, which was fed at the rate of 6½ pounds per day beginning 35 days after breeding. This was reduced to 5 pounds per head per day during parturition and during lactation the feed was increased to 10 pounds per day. It was reported that litter size was not affected, however, the livability and number of strong pigs appeared to be decreased with higher levels of nitrate. Milk production was not reduced as has been found in cattle. Serum nitrate of the control group was 1-3 mg. per 100 ml. and rose up to 16 mg. per 100 ml. in serum from the high nitrate feeding group.

Tollet et al. (1960) reported that $KNO_3$ levels of 1.84% or more in the growing ration depressed the growth rate of swine. In a second study they found that 3.68% of KCl in the ration, fed at iso-$K^+$ level to determine the effect of $K^+$, did not depress growth rate whereas both 5% $KNO_3$ and 4.35% $NaNO_3$ depressed growth rate. In a third study they fed 0.061, 1.84 and 3.17% of $NaNO_3$ with various levels of vitamin A (0, 1500 and 3600 I.U./lb. of feed). They found that 3.17% of $NO_3^-$ depressed growth and the methemoglobin level increased. In a study with gilts, Tollet et al. (1960) reported a depression of growth due to nitrate (0.61-3.17% of $KNO_3$ in total ration) but there were no differences in

number of corpora lutea, percent implantation, ovary weights, placental weights or thyroid weights when treated gilts were compared to controls.

O'dell et al. (1960) reported that it required only about 6 to 8 weeks to deplete vitamin A from the rats so that they were affected with xerophthalmia, when they were fed a diet containing 0.3% $KNO_2$. They reported that muscular incoordination similar to that of vitamin E deficiency was observed even though the diet contained adequate amounts of vitamin E. Appetite and muscular coordination were improved by supplementation with vitamin E. They also concluded that nitrite will prevent vitamin A storage when rats receive either vitamin A or carotene. Greathouse, Little and Mitchell (1962) fed rats $KNO_3$ and $KNO_2$ for 1 to 2 weeks. Twenty-four hours after the last feeding the rats were injected with beta-carotene and sacrificed three hours later to study the degree to which the carotene had disappeared from the intestine. Nitrate increased the carotene disappearance from the intestine. All the $NO_3^-$ in the intestine had disappeared within a 24 hour period. Two percent $KNO_3$ and 0.5% $KNO_2$ did not reduce feed consumption in that experiment.

Banji and Sundaresan (1961) fed a measured amount of retinene (vitamin A aldehyde) to control rats, thiourea-fed rats and iodinated casein-fed rats. They reported that retinene absorption was the same in all groups when sufficient time was allowed. Hyperthyroid rats showed a faster rate of absorption and had more vitamin A in the liver when the analysis was made two hours after the last treatment, however they showed less storage of liver vitamin A if the analysis was made five days after the last treatment. Conversely, the hypothyroid rats had consistently higher vitamin A reserves, probably due to poorer utilization of vitamin A.

Welsch et al. (1961), reported that rats fed 2.5% of $KNO_3$ in the diet developed larger adrenal and thyroid glands than those fed 1.8% of KCl. Emerick and Olson (1962) found that in the presence of 3% $NaNO_3$ and 0.5% $NaNO_2$ in the diet vitamin A palmitate dispersed in water contributed more to liver storage than vitamin A in oil and oral administration was better than injection. 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 not when vitamin A was subcutaneously injected. This suggests that the action of nitrite in reducing liver storage of the orally administered vitamin A was the result of either increased destruction in the digestive tract or a decrease in absorption from the intestine. Both nitrate and nitrite lowered the liver storage of vitamin A from carotene, with the greatest effect resulting from nitrite. It also was observed that sodium nitrite mixed with acid stomach contents destroyed carotene rapidly.

Winter (1962) reported that oral as well as parenteral administration of nitrate and nitrite resulted in wide variations in methemoglobin production in cattle. A nitrate drench was absorbed to a considerable extent and was metabolised to some extent in the tissues but very little in the blood. Nitrite and hydroxylamine were present in the blood in smaller amounts. Blood nitrite level and methemoglobin level were correlated to some extent. $NH_2OH$ is formed from $NO_2^-$ and it did convert hemoglobin to methemoglobin. Treatment with nitrate and nitrite had little effect on blood ammonia level.

Hale et al. (1962) reported a 56 day trial with cattle in which they fed 1% $KNO_3$ in concentrate at two levels of energy in a low carotene diet. The high energy ration resulted in a significantly larger depletion of the vitamin A level in the liver. One percent $KNO_3$ in the ration also reduced vitamin A stores but the reduction was not significant.

McLaren (1959) began depleting young rats of vitamin A while they were still with their mother at 16 days after birth. Vitamins A, D, and E were not given in his experiment. With 18 percent protein in the diet normal growth was obtained until the age of 60 days. 11 percent protein gave poor growth, 6 and 4 percent protein diet gave no growth. Rats receiving the lower protein developed xerophthalmia later than those having higher levels of protein, if they survived long enough. However, early deaths on the low protein diet in this experiment were thought to be due to avitaminosis E. Earlier depletion of vitamin A accompanied rapid growth. Mean survival time for the 18 percent protein group was 74 days, for the 11 percent protein it was 77.3 days. It appeared that vitamin A reserves were used at a rate proportionate to growth rate. The mean survival time for males was 56 days, for females 68 days. On the deficient diet mean survival time was 72 and 79 days respectively. On the low protein level which did not promote growth at all the females did not live longer than the males.

Johnson and Baumann (1947) produced hyperthyroid rats by feeding them desiccated thyroid tissues and hypothyroid by feeding thiouracil and thiourea. The rats were then fed either 40 mcg. of beta-carotene or 40 mcg. of vitamin A. They reported that when carotene was fed hyperthyroid rats stored more vitamin A in the liver than did the hypothyroid

rats. The administration of thyroxine neutralised the effect of thiourea and thiouracil, and restored the ability to convert carotene into vitamin A. An increase of metabolic activity resulted from feeding 2, 4 - dinitrophenol; however it was not accompanied by an increased rate of carotene conversion into vitamin A. It, therefore, was suggested that the altered carotene metabolism associated with thyroid disfunction was not due to changes in the basal metabolic rate per se, but was brought about by some other physiological action of the thyroid.

Serif and Brevik (1960) confirmed the report of Johnson and Baumann (1947) that there is an unequivocal relationship between thyroid status and the ability of animals to convert carotene to vitamin A. Hyperthyroid rats produced by feeding desicated thyroid had a higher total liver vitamin A content than control animals. They also reported that the form of thyroid hormone effective in the conversion of beta-carotene to vitamin A was tri-iodotyronine. Thyroxine per se, appeared to possess no activity in respect to the conversion of carotene to vitamin A.

Bloomfield et al. (1960) reported that the feeding of 0.31% and 0.92% of dietary nitrite affected iodine metabolism of the normal thyroid gland in both rats and sheep respectively. Frape et al. (1959a), studying the vitamin A requirement of young pigs reported that the requirement for vitamin A of young pigs appeared to be only slightly influenced by the environmental temperature. Frape et al. (1959b) also reported a study in which they injected $I^{131}$ into 83 day old pigs intraperitoneally and twenty hours later treated them with thiouracil. The pigs were kept at two environmental temperatures, 6° to 8°C and 18° to 21°C. The rate of thyroid secretion, plotted against the amount of vitamin A added in the feed at the rate of 0, 100, 400, 1600, and 6400 I.U./lb., had a maximum

at both temperatures at 100 I.U./lb. of feed. This level also gave the most efficient feed conversion in an earlier experiment. Thyroid secretion rate was less at the lower temperature with all vitamin A levels. In a second experiment they tried to relate growth rate and thyroid secretion rate but found that the relationship was rather small, therefore a more direct effect of vitamin A upon thyroid function was postulated. It also was concluded that within the range tested, dietary vitamin A had considerable influence upon the rate of thyroxine secretion. Insufficient or excessive intake of vitamin A lowered the rate of thyroid secretion. Average daily gain during the 13th week of life was maximum at the higher temperature when 400 I.U./lb. of vitamin A was added to each pound of feed and at lower temperature when 6400 I.U. of vitamin A was added to each pound of feed.

Cama et al. (1957) fed rats a vitamin A deficient diet plus iodinated casein, thiourea or thiouracil and gave them retinine orally or by injection or administered carotene orally. They reported that iodinated casein decreased and thiourea increased the storage of liver vitamin A from orally fed retinine. Both iodinated casein and thiourea slightly increased the liver storage from injected retinine but iodinated casein increased and thiourea decreased the liver storage when carotene was given orally.

Cama and Goodwin (1949) also reported, that in their studies with rabbits, thiouracil inhibited and desicated thyroid stimulated the absorption of beta-carotene from the intestinal tract. Desicated thyroid counteracted the effect of thiouracil when they were administered together. Neither of them had any qualitative effect on the stability of a colloidal solution of beta-carotene. Thiouracil itself did not seem to inhibit

intestinal "carotenase". They also suggested that the determination of plasma vitamin A levels are of little or no value in diagnosing thyroid disfunction.

Boguth and Sari (1962) removed the pituitaries of rats to inhibit thyroid function and depleted them for four weeks before giving each rat 100 mcg. of beta-carotene per day through a stomach tube. They found that signs of deficiency, xerophthalmia and muscular incoordination, disappeared within 4 to 5 days. Poor growth rate could be improved by subcutaneous injection of 1 mg. of testosterone propionate as well as by the administration of beta-carotene through stomach tube. As a result they concluded that the conversion of beta-carotene to vitamin A was independent of thyroid function.

Veil, Triantaphyllidis and Fornand (1961) reported that when vitamin A was given to rats at the rate of 5000, 20,000 and 30,000 I.U. per day through stomach tube for two weeks the basal metabolic rate was reduced 8.6, 12.1, and 11.4 percent respectively. The removal of the thyroid reduced basal metabolic rate a little over 30% and there was little change thereafter whether vitamin A was given or not. Excess vitamin A had little effect upon thyroid weight relative to body weight regardless of the iodine level of the diet. When labelled thyroxine was injected they reported that excess vitamin A caused the radioactive thyroxine to be more rapidly excreted in urine and feces. They concluded that the effect of excess vitamin A on basal metabolic rate was entirely mediated by thyroid.

Emerick and Lievan (1963) fed rats for 40 days on a vitamin A deficient diet containing $N_2O_4$ treated carotene and found that there were no toxic effects of $N_2O_4$ treated carotene but there was no vitamin A

activity left. There was no difference in weight gain at the same vitamin A activity level between the $N_2O_4$ treated carotene fed groups and the control fed groups.

Sell and Roberts (1963) fed growing chickens a ration containing 0.4% $KNO_2$. The $NO_2^-$ depressed growth under ad libitum feeding conditions by lowering the feed consumption. When the feed intake was equalized, the depression of growth could be observed only in those birds receiving the vitamin A free diet containing $NO_2^-$. Liver vitamin A was low in chickens fed either vitamin A or carotene along with nitrite. When vitamin A was injected the liver vitamin A stores were relatively high in the $NO_2^-$ treatment. Thyroid hypertrophy was observed in all chickens receiving nitrite. The enlargement of the thyroid could be overcome by massive doses of an intramuscular injection of vitamin A. Nitrite increased mortality but no vitamin A deficiency symptoms were observed during the study. They concluded that the influence of nitrite on feed intake and consequent growth of the chick resides in some other physiological process than that where either vitamin A or thyroid gland or both are critically involved.

Kuroksawa (1948) reported that environmental temperature may affect vitamin A depletion or the utilization of carotene or both. When animals were kept at a high temperature there was an increased requirement for vitamin A. This conclusion does not agree well with the results in swine reported by Frape et al. (1949b) who found that 400 I.U. of preformed vitamin A palmitate gave maximum growth at an environmental temperature of 18 to 20°C whereas 1600 I.U. and 6400 I.U. reduced growth rate, but at a temperature of about 6 to 8°C. Both 1600 I.U. and 6400 I.U. of vitamin A per pound of feed gave better growth.

Phillips (1962) reported the effect of environmental temperature on vitamin A and carotene metabolism in rats. He found that low temperature had no effect on either vitamin A or carotene depletion or utilization but it markedly increased the conversion of carotene into vitamin A. Comparing 2°C with 22°C for 21 days, he found that the rats on the vitamin A diet had more liver vitamin A when kept at the low temperature than those kept at the higher temperature. This was possibly due to higher feed consumption and therefore a greater vitamin A intake. Rats that were kept at 2°C and fed on beta-carotene had approximately twice the amount of liver vitamin A as those kept at 22°C on the beta-carotene diet. When vitamin A was orally administered there were no differences among rats kept at the different temperatures. In his second experiment the results were similar and it was found that liver storage of vitamin A in rats kept at low temperature was twice that of those kept at room temperature after only three days. Phillips (1962) explained the results as being due to the increase of thyroid activities during exposure to cold weather environment which in turn increased the conversion of carotene to vitamin A. The effect of exposure to cold on utilization of carotene still persisted when possible differences due to intestine microflora were eliminated by including sulphamerazine in the basal diet. It also was reported that low temperature did not increase the vitamin A requirement nor incidence of deficiency.

Porter and Masoro (1961) fed mature rats a semi-purified diet containing 34 I.U. of vitamin A per gram and kept them at 25°, 2° and 0°C. It was found that total liver vitamin A was twice as great as, and in I.U. per gram of liver was more than twice as great, for those kept at low temperature as for those at 25°C. They concluded that acclimatization to cold weather

does not have any sparing effect on vitamin A metabolism. Hjarde et al. (1961) also reported that environmental temperature had no effect on rate of vitamin A depletion. They compared a temperature of 1° to 2°C to a temperature of 14° to 16°C. This led to their conclusion that vitamin A requirement was not related to metabolic rate. This conclusion does not agree with that reported by Johnson and Baumann (1948).

Johnson and Baumann (1948) reported that rats whose growth was restricted by a low calorie diet, low thiamine diet or low tryptophan diet retained more hepatic vitamin A than control rats that grew normally. After comparing rats of similar size, they suggested that metabolic rate also influenced vitamin A depletion; desiccated thyroid hastened the depletion of vitamin A reserves while thiourea and thiouracil delayed it. They also observed that in normally growing rats, a decrease in hepatic vitamin A reserves was accompanied by an increase in the amount and concentration of vitamin A in the kidneys. No such increase occurred in rats whose growth rate was retarded during the depletion period. The concentration of vitamin A in kidney was particularly low in rats exposed to thiourea.

## METHODS

Two separate trials in which various levels of sodium nitrate or sodium nitrite were mixed in feed fed to growing swine were completed. In the first trial 0.60 and 1.20 percent of $NaNO_3$, raised to 1.0 and 2.0 percent respectively after four weeks were added to rations that were either normal (approximately 400 I.U. of vitamin A activity) or low (approximately 23 I.U. of vitamin A activity/lb.) in vitamin A content. (ration 29D and ration 29A, Table 1). Two levels of $NaNO_2$, 0.15 and 0.30

percent, (raised to 0.20 and 0.40 percent after four weeks) were added to the 29D ration only. Fourteen weanling pigs, Duroc and crossbred (Duroc X Poland China), gilts and barrows, were randomized according to weight, breed and sex and allotted into 7 groups as follows:

Group 1 Positive control - (ration 29D)
2 29D + 0.60% $NaNO_3$
3 29D + 1.20% $NaNO_3$
4 29D + 0.15% $NaNO_2$
5 29D + 0.30% $NaNO_2$
6 Negative control - (ration 29A)
7 29A + 0.60% $NaNO_3$

The first trial was carried on during the summer from June 10, 1962 to August 29, 1962. The total time involved was 71 days. Venous blood samples were collected from the anterior vena cava at intervals to determine levels of hemoglobin and methemoglobin. Serum vitamin A was determined at the end of the trial. At the conclusion of the trial all pigs were put together on pasture on normal diets and blood samples were collected again 20 days later to study the repletion of serum vitamin A. Feed consumption and average daily gain were determined weekly.

Twenty vitamin A depleted Poland China pigs, obtained by feeding both the sows and their litters a low vitamin A activity diet throughout the gestation and suckling period until the pigs were weaned and put into the experiment, were used in the second study. Pigs were randomized to sex, weight and litter into 10 groups of 6 treatments as follows:

Group 1 Control, ration (29E)
2 29E + 3% $NaNO_3$
3 29E + 5% $NaNO_3$
4 29E + 0.3% $NaNO_2$
5 29E + 0.5% $NaNO_2$
6 29E + 29A (1:1) + 3% $NaNO_3$
7 29E + 5% $NaNO_3$
8 29E + 0.5% $NaNO_2$
9 29E + 29A (1:1) + 3% $NaNO_3$
10 Control, ration (29E)

Table 1. Rations used in the experiment.

| Ingredient (%) | 29A | 29D | 29E |
|---|---|---|---|
| Soybean oilmeal | 13.2 | 13.2 | 13.2 |
| Sorghum grain | 81.2 | - | - |
| Yellow corn | - | 81.2 | 76.2 |
| Alfalfa meal | - | 1.0 | 5.0 |
| Dried skim milk | 2.5 | 2.5 | 2.5 |
| Brewers' yeast | 1.0 | 1.0 | 1.0 |
| Iodised salt | 0.4 | 0.4 | 0.4 |
| Bone meal | 0.4 | 0.4 | 0.4 |
| Trace minerals[1] | 0.05 | 0.05 | 0.05 |
| Vitamin B complex[2] | + | + | + |
| Total | 100 | 100 | 100 |
| Estimated vitamin A activities, I.U./lb.[3] | less than-23 | 400-800 | 2,000-4,000 |

[1] Containing Fe 10%, Mn 10%, Cu 1%, Co 0.1%, I 0.3%, and Zn 5%.

[2] Supplying the following vitamins per lb. of feed: choline 10 mg., niacin 3 mg., riboflavin 1 mg. and calcium pantothenate 2 mg.

[3] Using estimated activity because the potency of carotene between samples of alfalfa meal varied markedly. One alfalfa meal analysed early in winter contained 82,300 I.U. of vitamin A activity per pound.

No preformed vitamin A added in these rations.

The second experiment lasted from December 7, 1962 to March 26, 1963 (81 days). Carotene analyses showed that ration 29E was high in vitamin A activity. The lower vitamin A activity ration which was fed to groups 6 and 9 was obtained by mixing ration 29A and 29E in the ratio of 1:1 by weight. The lower vitamin A activity ration was included to study the effect of different vitamin A activity levels in feed during the winter trial. Pigs were kept in an unheated barn and so they were exposed to rather cold temperatures during the winter months. Venous blood was collected at intervals from the anterior vena cava for determination of serum vitamin A, hemoglobin and methemoglobin. At the end of the study one-half the pigs were slaughtered after the supplemented feeds had been replaced by the control ration (29E) for a period of approximately 30 hours. Hemoglobin, methemoglobin, serum and liver vitamin A, nitrate and nitrite in blood and in muscle and fat from ham and loin were determined after slaughter. The remaining pigs were fed both ration 29A and 29E for a period of 25 days during recovery and were then slaughtered. Again, serum vitamin A, liver vitamin A, hemoglobin and methemoglobin were determined.

Basal or control feeds used in the experiment were mixed and sacked in 50 pound paper bags by the Feed Milling Department. A 200 pound double-ribbon horizontal mixer was used to mix nitrate and nitrite into the various feeds. The particle size of both chemicals was reduced to the approximate fineness of table salt before they were mixed into the feed. Before mixing in the mixer, the chemical, nitrate or nitrite, was premixed with an appropriate amount of feed by hand to insure the proper distribution in the feed. Feed was given ad libitum in a self-feeder. The pigs were watered two or three times per day by hand.

Serum and liver vitamin A was determined by Kimble's method (1939). Hemoglobin and methemoglobin were determined by Wong's method (Hawk, Oser and Summerson, 1947). Nitrate in blood and tissues was determined by the diphenylamine test (Jacobs, 1958), the nitrite by the color reaction with N-(1 naphthyl) - ethylene diamine dihydrochloride as described by Diven et al. (1961).

Preparation of blood for the determination of nitrate and nitrite was done according to the method described by Diven et al. (1961). For fat, muscle and liver, approximately 50 gm. of the tissues were extracted by hot water six times. The water extracts obtained were then evaporated down to 25 ml. for fat and muscle and 50 ml. for liver. The evaporated water extracts were used in the determination of nitrate and nitrite.

## RESULTS AND DISCUSSION

Results measured in terms of average daily gain, feed consumption and feed conversion efficiency for experiments 1 and 2 are summarized in Tables 2 and 4. In the first experiment, differences in growth rate were not significant. However, it was observed that pigs in both nitrite groups had a tendency to consume less feed and grow more slowly during the second period of 6 weeks.

In the second experiment, which was carried out during cold winter weather, significant differences in growth rate were obtained ($P<.05$), (Tables 4 and 5). Pigs eating the 3% $NaNO_3$ ration (treatment 2 and 3) made an average daily gain only approximately one-half as great as those eating the control ration and pigs eating the 5% $NaNO_3$ ration (treatment 4) gained only one-fifth as much as the controls. Reduction of vitamin A activity to about fifty percent (treatment 3) resulted in higher feed

Table 2. Summary of results for average daily gain, average daily feed consumption and average feed conversion efficiency (lb. of feed per lb. of gain) in experiment 1.

| Treatment<br>Ration & supplementation | 1<br>29D | 2<br>29D + 0.6% $NaNO_3$ | 3<br>29D + 1.2% $NaNO_3$ | 4<br>29D + .15% $NaNO_2$ | 5<br>29D + .3% $NaNO_2$ | 6<br>29A | 7<br>29A + 0.6% $NaNO_3$ |
|---|---|---|---|---|---|---|---|
| Number of pigs | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| Average weight, lb. | | | | | | | |
| initial | 66.5 | 68.5 | 73.5 | 61.0 | 68.5 | 66.0 | 66.5 |
| end of 4th week | 122.0 | 112.5 | 125.5 | 104.5 | 111.5 | 113.0 | 111.0 |
| end of 10th week | 180.5 | 185.0 | 195.5 | 163.5 | 166.0 | 172.0 | 171.5 |
| Average daily gain, lb. | | | | | | | |
| first 4 weeks | 1.63 | 1.57 | 1.86 | 1.55 | 1.54 | 1.71 | 1.59 |
| second 6 weeks | 1.82 | 1.73 | 1.67 | 1.39 | 1.29 | 1.40 | 1.44 |
| overall period | 1.74 | 1.66 | 1.74 | 1.46 | 1.39 | 1.57 | 1.50 |
| Average daily feed consumption, lb. | | | | | | | |
| first 4 weeks | 4.31 | 4.09 | 5.29 | 3.85 | 4.07 | 4.40 | 4.21 |
| second 6 weeks | 5.61 | 5.64 | 5.67 | 4.15 | 4.41 | 4.85 | 4.98 |
| overall period | 5.02 | 5.02 | 5.51 | 4.03 | 4.28 | 4.60 | 4.67 |
| Average feed efficiency | | | | | | | |
| first 4 weeks | 2.65 | 2.60 | 2.85 | 2.48 | 2.65 | 2.57 | 2.65 |
| second 6 weeks | 3.16 | 3.27 | 3.40 | 2.98 | 3.39 | 3.43 | 3.45 |
| overall period | 2.88 | 3.20 | 3.16 | 2.76 | 3.07 | 2.93 | 3.11 |

During the second period of six weeks amounts of sodium nitrate and sodium nitrite supplemented in treatments 2, 3, 4, 5 and 7 were increased to 1.0, 2.0, 0.2, 0.3 and 1.0% of the ration respectively.

Table 3. Analysis of variation for average daily gain in experiment 1.

| Source of variation | d.f. | M.S. |
|---|---|---|
| Treatment | 6 | .03967 |
| Sex | 1 | .00133 |
| Error | 5 | .03492 |

Table 4. Summary of results for average daily gain, average daily feed consumption and average feed efficiency in experiment 2.

| Treatment<br>Ration and supplementation | 1<br>29E | 2<br>29E + 3% $NaNO_3$ | 3<br>29A + 29E (1:1)+3% $NaNO_3$ | 4/1<br>29E + 5% $NaNO_3$ | 5<br>29E + 0.3% $NaNO_2$ | 6<br>29E + 0.5% $NaNO_2$ |
|---|---|---|---|---|---|---|
| Number of pigs | 4 | 2 | 4 | 2 | 2 | 4 |
| Average weight, lb. | | | | | | |
| initial | 55.0 | 50.5 | 54.5 | 68.0 | 46.0 | 57.4 |
| final | 150.8 | 99.0 | 105.8 | 78.0 | 120.5 | 107.3 |
| Average daily gain, lb. | 1.18 | 0.60 | 0.63 | 0.25 | 0.82 | 0.62 |
| Average daily feed consumption | 4.86 | 2.98 | 3.72 | 4.90 | 3.71 | 3.16 |
| Average feed efficiency | 4.11 | 4.98 | 5.88 | 89.70 | 4.03 | 5.12 |

/1In treatment 4, only the weights of two pigs were averaged. The pigs that died early in the trial were not used in the calculations.

Table 5. Analysis of variation for average daily gain on experiment 2.

| Source of variation | d.f. | M.S. |
|---|---|---|
| Treatment | 5 | .26921* |
| Sex | 1 | .02133 |
| Error | 11 | .06947 |

*P<.05

consumption and better average daily gain but poorer feed efficiency than when pigs were fed 3% $NaNO_3$ (treatment 2) during extremely cold weather. Pigs on both nitrite levels also grew slower than the controls but faster than those on nitrate. Growth rate decreased as levels of nitrate or nitrite in the ration were increased. Growth depressing effects of nitrite could have been primarily due to reduced feed intake. Three-tenths percent $NaNO_2$ in the ration brought about better feed conversion efficiency than the control ration even though the pigs grew slower and consumed less feed per day. When dietary nitrite was increased to 0.5% of the total ration (treatment 6) pigs grew more slowly, consumed less feed and had poorer feed conversion efficiency. A pig fed the 5% $NaNO_3$ ration developed a habit of using his fore-feet to pull the feed out of the feeder on to the wet floor which may reflect unpalatability and unacceptability of feed containing excessive amounts of nitrate. Such feed wastage could not be measured accurately by any means, therefore, feed consumption and feed efficiency figures for treatment 4 have little meaning.

Chronic diarrhea was observed in all nitrate treatment groups during the summer (experiment 1). Diarrhea seemed to be more prevalent and severe after rainfall. In experiment 1, all pigs were in good health and showed no sign of weakness nor any visible symptoms of methemoglobinemia. Diarrhea was not observed during the second experiment, possibly because the pigs ate less feed and drank less water. Dehydration of body fluids during the winter experiment was indicated by the rise of hemoglobin level above the normal level in all treatments including the control, with the exception of the nitrite treatments (treatment 5 and 6). Two pigs fed the 5% $NaNO_3$ ration died. The first one died suddenly and symptoms before

death were not observed. The second one had stiff legs and neck, muscular incoordination, was unable to stand and died about 18 hours after symptoms were observed. Post morten examination showed that all tissues and blood were chocolate brown in color. Catarrhal enteritis and hyperemia were found in the intestinal tracts.

In both experiments consumption of added nitrite in feed lowered hemoglobin content significantly while consumption of added nitrate did not. It can be seen from Table 6 and Table 9 that all levels of dietary nitrite had a similar effect on hemoglobin level. The average values for hemoglobin in experiment 1 were 12.5 and 13.3 gm. per 100 ml., and in experiment 2 they were 13.0 and 13.3 gm. per 100 ml. for the lower and the higher nitrite levels respectively. The hemoglobin level measured at different times in the same pig, after supplementation with nitrite, fluctuated slightly but rarely fell below 12 gm./100 ml. Pigs eating the lower nitrite ration in experiment 1 showed no significant increase of methemoglobin level but the serum vitamin A level was lower than that of negative controls (treatment 6) and they still had a hemoglobin level as low as those eating the higher nitrite ration which had an increased methemoglobin level. This result might indicate that the animal body has a mechanism to get rid of $NO_2^-$ in vivo at the expense of hemoglobin. This mechanism is limited and after that limit is reached the $NO_2^-$ entering the body will then combine with hemoglobin and form methemoglobin which will increase as the level of $NO_2^-$ entering the blood circulation is increased. It was observed in the second experiment that there was dehydration of body fluid in all treatments. The hemoglobin level rose even in the controls but dietary nitrite still exerted its effect by lowering hemoglobin level down to 13 gm./100 ml. (Table 9).

Table 6. Effect of added dietary nitrate and nitrite on serum vitamin A (mcg./100 ml.) hemoglobin and methemoglobin (Hb. and Methb. in gm./100 ml.) in experiment 1.

| Treatment | Before supplementation | | | | After supplementation | | | | | | | | | | | | Serum vitamin A | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Breed-Sex [1] | 6/20/1962 Hb. | Methb. | % [2] Methb. | 6/26/1962 Hb. | Methb. | 7/11/1962 Hb. | Methb. | 7/26/1962 Hb. | Methb. | 8/29/1962 Hb. | Methb. | Average Hb. | Methb. | % [2] Methb. | End of 20 days of Expt. | 20 days of repletion |
| 1 | D-G | 16.2 | .24 | 1.48 | 13.3 | .53 | 16.6 | .53 | 15.3 | .33 | 15.8 | .64 | 15.3 | .51 | 3.33 | 15.62 | 13.98 |
| | X-B | 12.8 | .14 | 1.13 | 13.8 | .58 | 17.6 | .66 | 18.6 | .29 | 18.1 | .54 | 17.0 | .51 | 3.16 | 24.48 | 24.93 |
| 2 | D-B | 15.0 | .19 | 1.27 | 6.2 | .41 | 14.4 | .57 | 13.8 | .24 | 14.5 | .53 | 14.2 | .44 | 2.32 | 6.04 | 18.11 |
| | X-G | 16.1 | .14 | .87 | 6.9 | .61 | 15.7 | .48 | 13.1 | .28 | 14.4 | .43 | 14.3 | .45 | 2.72 | 13.98 | 18.52 |
| 3 | D-G | 17.0 | .24 | 1.41 | 10.3 | .47 | 17.5 | .39 | 17.6 | .29 | 17.5 | .49 | 15.7 | .41 | 2.80 | 11.56 | 14.09 |
| | X-B | 15.3 | .28 | 1.83 | 13.1 | .19 | 16.2 | .11 | 17.3 | .33 | 15.7 | .58 | 15.6 | .30 | 1.93 | 10.74 | 23.07 |
| 4 | D-G | 15.6 | .24 | 1.53 | 15.2 | .53 | 13.6 | - | 12.8 | .38 | 10.5 | 1.30 | 13.0 | .73 | 6.24 | 0.38 | 19.78 |
| | X-B | 13.1 | .38 | 2.90 | 12.8 | - | 11.9 | .38 | 11.7 | .28 | 11.8 | .53 | 12.1 | .40 | 3.32 | 3.75 | 21.48 |
| 5 | D-G | 11.6 | .22 | 1.90 | 13.6 | 3.87 | 12.0 | 3.76 | 11.7 | 3.10 | 10.1 | 2.72 | 12.1 | 3.36 | 27.55 | 0.00 | 18.11 |
| | X-B | 12.6 | .23 | 1.83 | 14.6 | 4.30 | 14.0 | 3.19 | 14.6 | 3.65 | 13.2 | 2.22 | 14.4 | 3.34 | 23.51 | 4.13 | 19.29 |
| 6 | D-B | 14.6 | .47 | 3.21 | 13.3 | .24 | 16.3 | .56 | 19.0 | - | 18.0 | .44 | 16.8 | .41 | 2.58 | 6.04 | 17.66 |
| | X-G | 15.7 | .24 | 1.53 | 15.2 | .38 | 16.1 | .43 | died 7/11/62 | | | | 15.7 | .40 | 2.59 | - | - |
| 7 | D-B | 13.1 | .23 | 1.76 | - | .27 | 16.7 | - | 17.0 | .38 | 16.7 | .44 | 16.8 | .41 | 2.43 | 8.01 | 19.78 |
| | X-G | 14.8 | .57 | 3.85 | 15.2 | .48 | 12.1 | .37 | 18.7 | .34 | 17.9 | .44 | 15.8 | .41 | 2.62 | 6.41 | 22.34 |

[1] D = Duroc Jersey, X = crossbred, G = gilt, B = barrow. Missing values caused by clotting of blood samples during collection. In analysis of variation, the % of methemoglobin in total hemoglobin was used.

[2] is the methemoglobin level expressed as percentage of total hemoglobin.

Table 7. Analysis of variance for hemoglobin and methemoglobin in experiment 1.

| Source of variation | Hemoglobin d.f. | Hemoglobin M.S. | Methemoglobin d.f. | Methemoglobin M.S. |
|---|---|---|---|---|
| Treatment | 6 | 18.29 | 6 | 470.77* |
| Sampling[1] | | | | |
| a. | 1 | 1.22 | 1 | 275.64* |
| b. | 3 | 77.00 | 3 | 9.79 |
| Error (a) | 23 | 5.27 | 22 | 11.38 |
| Breed | 1 | 6.03 | 1 | 19.76 |
| Breed X treatment | 6 | 2.73 | 6 | 4.42 |
| Error (b) | 24 | 1.96 | 21 | 27.96 |

*$P < .005$

[1]Sampling a. is the difference between samples collected before the pigs were fed with rations supplemented with nitrate or nitrite and the samples collected after supplementation. Sampling b. is the difference among the samples collected at different times after starting the feeding of supplemented rations.

Table 8. Duncan's new multiple range test for hemoglobin and methemoglobin level in experiment 1.

1. Hemoglobin level

| Treatment | 6 -control | 1 +control | 3 1.2% nitrate | 7 0.6% nitrate | 2 0.6% nitrate | 4 0.15% nitrite | 5 0.3% nitrite |
|---|---|---|---|---|---|---|---|
| No. of observations | 8 | 10 | 10 | 9 | 8 | 10 | 10 |
| mean | 16.00 | 15.86 | 15.75 | 15.69 | 14.63 | 12.90 | 12.80 |

2. Methemoglobin level

| Treatment | 3 1.2% nitrate | 2 0.6% nitrate | 7 0.6% nitrate | 6 -control | 1 +control | 5 .15% nitrite | 4 0.3% nitrite |
|---|---|---|---|---|---|---|---|
| No. of observations | 10 | 8 | 7 | 8 | 10 | 8 | 10 |
| mean | 2.22 | 2.45 | 2.52 | 2.61 | 2.84 | 4.16 | 21.10 |

Table 9. Effect of dietary nitrate and nitrite on hemoglobin and methemoglobin levels (Hb. and Methb. in gm./100 ml.) in experiment 2.

| Treatment, Litter and Sex | Before supplementation 12/7/62 Hb. | Methb. | %/1 Methb. | After supplementation 1/9/63 Hb. | Methb. | 1/26/63 Hb. | Methb. | 2/26/62 Hb. | Methb. | Average Hb. | Methb. | %/1 Methb. | Slaughtered 2/27-28/63 Hb. | Methb. | %/1 Methb. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 Control | | | | | | | | | | | | | | | |
| 15-B | 22.3 | .19 | 0.85 | 17.9 | .68 | 19.0 | .54 | 21.0 | .35 | 19.3 | .52 | 2.58 | 22.6 | .15 | 0.66 |
| 14-G | 16.7 | .48 | 3.47 | 18.1 | .53 | 20.4 | .49 | 21.1 | .24 | 19.9 | .42 | 2.17 | 23.2 | .69 | 2.97 |
| 13-B | 13.3 | .56 | 4.21 | 17.7 | .86 | 19.5 | .59 | 20.4 | .20 | 17.3 | .55 | 3.29 | | | |
| 14-G | 14.8 | .38 | 2.36 | 17.0 | .78 | 18.7 | .53 | 19.9 | .20 | 18.5 | .50 | 2.77 | | | |
| 2 3% $NaNO_3$ | | | | | | | | | | | | | | | |
| 18-B | 10.0 | .33 | 3.20 | 10.4 | .42 | 11.6 | .54 | 13.6 | .44 | 11.9 | .47 | 3.95 | 8.4 | .37 | 4.60 |
| 20-G | 15.7 | .37 | 2.31 | 16.9 | .63 | 19.8 | .63 | 22.0 | .30 | 19.6 | .53 | 2.75 | | | |
| 3 3% $NaNO_3$ + carotene | | | | | | | | | | | | | | | |
| 13-B | 14.2 | .28 | 1.97 | 17.8 | .39 | 20.4 | .34 | 22.0 | .39 | 20.1 | .37 | 1.87 | 16.2 | .52 | 3.21 |
| 20-G | 15.1 | .33 | 3.50 | 19.2 | .43 | 23.3 | .05 | 20.9 | .39 | 21.1 | .29 | 1.43 | 23.1 | .29 | 1.26 |
| 14-B | 16.2 | .33 | 2.03 | 19.3 | .63 | 22.0 | .44 | 22.0 | .30 | 21.1 | .46 | 2.21 | | | |
| 18-G | 14.4 | .28 | 1.94 | 18.7 | .63 | 20.2 | .59 | 19.9 | .29 | 19.6 | .50 | 2.58 | | | |
| 4 5% $NaNO_3$ | | | | | | | | | | | | | | | |
| 13-B | 16.3 | .43 | 2.63 | 22.3 | .53 | 21.7 | .60 | 21.4 | .63 | 21.8 | .59 | 2.69 | 18.1 | .15 | 0.83 |
| 14-G | 13.7 | .33 | | | | | | | | | | | | | |
| 15-B | 13.9 | .28 | 2.01 | 19.1 | .49 | 22.3 | .54 | 20.5 | .24 | 20.6 | .42 | 2.05 | | | |
| 20-G | 13.8 | .14 | | | | | | | | | | | | | |
| 5 0.3% $NaNO_2$ | | | | | | | | | | | | | | | |
| 15-B | 15.1 | .19 | 1.25 | 15.2 | 1.38 | 15.2 | 1.51 | 14.4 | 1.71 | 14.9 | 1.53 | 10.27 | 13.9 | .52 | 3.74 |
| 12-G | 13.9 | .43 | 3.09 | 11.0 | 1.06 | 10.9 | 1.27 | 11.3 | .48 | 11.1 | .94 | 8.48 | | | |
| 6 0.5% $NaNO_2$ | | | | | | | | | | | | | | | |
| 20-B | 16.2 | .14 | 0.86 | 14.9 | 3.77 | 14.2 | 2.23 | 15.6 | 6.34 | 15.2 | 4.11 | 26.87 | | | |
| 15-G | 14.2 | .24 | 1.69 | 12.3 | 3.82 | 12.4 | 2.05 | 12.3 | 3.81 | 12.3 | 3.23 | 28.03 | | | |
| 6-B | 15.3 | .33 | 2.16 | 12.4 | 3.72 | 13.2 | 3.34 | 12.6 | 4.17 | 12.7 | 3.74 | 29.47 | 12.9 | .83 | 6.43 |
| 12-G | 13.5 | .52 | 3.85 | 13.4 | 4.07 | 12.5 | 2.27 | 12.8 | 4.63 | 12.9 | 3.66 | 28.23 | 12.4 | .84 | 6.77 |

/1 Expressed as percentage of total hemoglobin.

Table 10. Analysis of variation for hemoglobin, methemoglobin and serum vitamin A in experiment 2.

| Source of variation | d.f. | Hemoglobin M.S. | Methemoglobin M.S. | Serum vitamin A M.S. |
|---|---|---|---|---|
| Treatment | 5 | 93.63 | 877.83** | 339.69** |
| Sampling[1] | | | | |
| a. | 1 | 63.05 | 580.99 | 94.78* |
| b. | 2 | 13.15 | 42.04 | 32.62 |
| Treatment X sampling | 15 | 88.81 | 136.43 | 14.97 |
| Sex | 1 | 6.27* | 15.85 | 8.51 |
| Sex X treatment | 5 | 34.49** | 1.11 | 82.35** |
| Error | 42 | 1.51 | 5.25 | 15.33 |

*P<.05
**P<.005

[1]Sampling a. is the difference between samplings collected before the pigs were fed with rations supplemented with nitrate or nitrite and the samplings collected after supplementation. Sampling b. is the difference among the samplings collected at different times after the pigs were fed with supplemented ration.

Table 11. Duncan's new multiple range test for hemoglobin, methemoglobin and serum vitamin A in experiment 2.

1. Hemoglobin levels

| Treatment | 4<br>5% nitrate | 3/1<br>3% nitrate | 1<br>control | 2<br>3% nitrate | 6<br>0.5% nitrite | 5<br>0.3% nitrite |
|---|---|---|---|---|---|---|
| No. of observations | 8 | 16 | 16 | 8 | 16 | 8 |
| mean | 19.7 | 19.1 | 18.3 | 15.1 | 13.7 | 13.4 |

2. Methemoglobin levels

| Treatment | 3/1<br>3% nitrate | 4<br>5% nitrate | 1<br>control | 2<br>3% nitrate | 5<br>0.3% nitrite | 6<br>0.5% nitrite |
|---|---|---|---|---|---|---|
| No. of observations | 16 | 8 | 16 | 8 | 8 | 16 |
| mean | 2.11 | 2.36 | 2.73 | 3.22 | 7.58 | 21.65 |

3. Serum vitamin A

| Treatment | 1<br>control | 3/1<br>3% nitrate | 2<br>3% nitrate | 4<br>5% nitrate | 5<br>0.3% nitrite | 6<br>0.5% nitrite |
|---|---|---|---|---|---|---|
| No. of observations | 16 | 16 | 8 | 8 | 8 | 16 |
| mean | 22.43 | 18.39 | 17.05 | 17.00 | 12.80 | 8.73 |

1/ is the 3.0% sodium nitrate supplemented in approximately 50% vitamin A activity level ration.

Table 12. Effect of nitrate and nitrite on serum vitamin A (microgram/100 ml.) and liver vitamin A (mcg/gm. wet weight) in experiment 2.

| Treatment Litter and Sex | Weight, lbs. 12/7 initial | 2/26 final | 3/23 | Serum vitamin A Before suppl. 12/7/62 | after supplementation 1/9/63 | 1/26/63 | 2/26/63 | liver vit. A 2/27-28 | 3/23/1963 serum vitamin A | liver vitamin A |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 Control | | | | | | | | | | |
| 15-B | 62 | 164 | | 20.2 | 16.9 | 23.6 | 21.5 | 23.9 | | |
| 14-G | 59 | 145 | | 29.8 | 25.8 | 22.3 | 19.4 | 26.0 | | |
| 13-B | 62 | 171 | 225 | 18.9 | 26.4 | 23.6 | 21.5 | - | 19.7 | 22.2 |
| 14-G | 37 | 123 | 175 | 18.1 | 24.9 | 21.9 | 24.1 | - | 17.2 | 22.5 |
| 2 3% $NaNO_3$ | | | | | | | | | | |
| 18-B | 47 | 74 | | 13.2 | 14.4 | 11.2 | 16.0 | 16.1 | | |
| 20-G | 54 | 124 | 179 | 18.1 | 21.9 | 16.8 | 24.1 | | 16.8 | 4.6 |
| 3 3% $NaNO_3$ ½ carotene | | | | | | | | | | |
| 13-B | 42 | 96 | | 24.5 | 14.0 | 15.9 | 28.0 | 9.6 | | |
| 20-G | 63 | 132 | | 14.0 | 18.1 | 19.8 | 15.6 | 10.3 | | |
| 14-B | 55 | 93 | 141 | 27.6 | 22.7 | 13.6 | 21.5 | | 21.4 | 10.1 |
| 18-G | 58 | 102 | 150 | 16.0 | 15.6 | 17.2 | 12.0 | | 15.2 | 9.1 |
| 4 5% $NaNO_3$ | | | | | | | | | | |
| 13-B | 79 | 88 | | 19.8 | 20.6 | 17.2 | 25.8 | 11.1 | | |
| 14-G | 34 | - | | 13.6 | died 21/12/62, at 42 lbs. | | | | | |
| 15-B | 57 | 68 | 127 | 17.2 | 15.6 | 15.6 | 4.1 | | 10.4 | 10.2 |
| 20-G | 42 | | | 19.4 | died 27/12/62 | | | | | |
| 5 0.3% $NaNO_2$ | | | | | | | | | | |
| 15-B | 62 | 152 | | 14.4 | 11.6 | 6.4 | 10.7 | 8.1 | | |
| 12-G | 30 | 89 | 140 | 22.3 | 12.3 | 8.4 | 14.8 | | 16.0 | 11.2 |
| 6 0.5% $NaNO_2$ | | | | | | | | | | |
| 20-B | 46 | 104 | 166 | 12.0 | 8.8 | 2.6 | 6.8 | | 12.3 | 10.1 |
| 15-G | 60 | 105 | 156 | 6.8 | 4.5 | 5.3 | 12.0 | | 13.9 | 6.6 |
| 6-B | 60 | 105 | | 14.4 | 8.8 | 4.5 | 6.4 | 4.5 | | |
| 13-G | 64 | 115 | | 18.9 | 13.2 | 8.0 | 6.8 | 6.8 | | |

The effect of dietary nitrite on methemoglobin level seemed equivocal when the results from the two experiments were compared. Computing the amount of nitrite intake per pig per day indicates that methemoglobin level increases as the amount of dietary nitrite intake increases.

The additive effect of $NO_2^-$ on methemoglobin level can be clearly seen when the two experiments are compared by computing methemoglobin in the second experiment from the figures obtained from experiment 1 (Table 13). From direct comparison, the difference between methemoglobin level in experiment 2 should be

$$\frac{(25.53 - 4.78)}{(57.593 - 28.76)} \times (87.070 - 60.698) = 18.627$$

which does not deviate far from the true difference obtained of 18.76 (28.14 - 9.38), Table 13.

The differences between methemoglobin levels in the two experiments confirm the conclusion, stated earlier, that the animal has a mechanism to get rid of $NO_2^-$ in vivo at the expense of hemoglobin. From Table 13, it can be seen that a daily intake of $NaNO_2$ of 57.953 mg./lb. body weight caused a rise in the methemoglobin level to 25.53% of the total hemoglobin. During the winter experiment (experiment 2) daily intake of $NaNO_2$ of 60.698 mg. and 87.070 mg. per pound body weight caused a rise to only 9.38 and 28.15% respectively. It is suggested that during the winter experiment there was a dehydration of body fluid which caused a rise in the hemoglobin level. The hemoglobin level was thereby increased and apparently the ability of the animal to get rid of $NO_2^-$ at the expense of hemoglobin was increased accordingly. The amount of excess $NO_2^-$ entering the circulation to be bound in methemoglobin was decreased because it was immediately destroyed at the expense of hemoglobin. This

Table 13. Relationship between dietary nitrite and methemoglobin level.

| | Av. $NaNO_2$ per pig daily gm. | Av. $NaNO_2$ per lb. body weight daily (1) mg. | mM. | Av. Methb., % of total Hb. |
|---|---|---|---|---|
| Experiment 1 | | | | |
| Treatment 4 (0.15 - 0.2% $NaNO_2$) | 3.2007 | 28.576 | 0.4141 | 4.78 |
| Treatment 5 (0.3 - 0.4% $NaNO_2$) | 6.8009 | 57.953 | 0.8399 | 25.53 |
| Experiment 2 | | | | |
| Treatment 5 (0.3% $NaNO_2$) | 5.0530 | 60.698 | 0.8797 | 9.38 |
| Treatment 6 (0.5% $NaNO_2$) | 7.1732 | 87.070 | 1.2619 | 28.14 |

(1) Obtained by dividing av. $NaNO_2$ per pig per day by (initial wt. + final wt.)/2

mechanism would be rapid and dynamic or else in lowering of hemoglobin without the significant production of methemoglobin would not have occurred in the first experiment. If we assume that the pig eats feed at a uniform rate from the self-feeder $NO_2^-$ would enter into the circulation at a constant rate, and cause a persistent low hemoglobin level in addition to causing uniformity of methemoglobin levels in all the blood samplings collected at different times. The rapid decrease of methemoglobin within the period of 30 hours after the nitrite containing rations were replaced by the non-nitrite control ration at the end of experiment 2, may indicate that there is a rapid rate of $NO_2^-$ turn over in vivo. The fluctuation in methemoglobin values which can be observed was small and was apparently a normal fluctuation for methemoglobin levels in this experiment.

If it is assumed that the capability of the animal body to detoxicate $NO_2^-$ without the formation of methemoglobin depends upon the hemoglobin level being higher than 12-13 gm./100 ml., the rise of methemoglobin, a sign of $NO_2^-$ toxicity, is a conditioned effect because the hemoglobin level will vary both due to individual variation and to environmental variation.

Because of the small amount of data obtained in these studies the exact linear regression equation relating the amount of nitrite intake per unit body weight and the percentage increase of methemoglobin could not be determined. The pattern of nitrite ions in vivo should roughly follow this model:

$$I = k.(Hb_2 - Hb_1) + M$$

When I = daily intake of $NO_2^-$ in millimole per pound body weight.

k = amount of $NO_2^-$ in mM. that can be handled by the animal mechanism at the expense of 1 unit gm./100 ml. of hemoglobin.

$Hb_2$ = average normal hemoglobin level, expressed as gm./100 ml. in that population under that environmental condition.

$Hb_1$ = Hemoglobin level at which the individual animal mechanism becomes limiting.

M = amount of $NO_2^-$ in mM. which is bound in methemoglobin. $k(Hb_2 - Hb_1)$ is then the amount of $NO_2^-$ which is handled by the animal's mechanism at the expense of hemoglobin.

If it is assumed that the excess $NO_2^-$ which cannot be handled by the animal's mechanism has an additive effect on methemoglobin formation, the level of methemoglobin, expressed as % of total hemoglobin, that will be produced from 1 mM. of $NO_2^-$ can be estimated from experiment 2 to be:

$$\% \text{ Methb.} = \frac{28.14 - 9.38}{1.2619 - 0.8797} = 49.11\%$$

The average values of hemoglobin for pigs that did not have nitrite added to the feed were calculated to be 15.7 and 19.1 gm/100 ml. for experiment 1 and experiment 2 respectively.

In the same way normal methemoglobin levels for experiment 1 and experiment 2 were 2.65% and 2.5% of Hb, respectively.

The average hemoglobin levels for low and high nitrite treatments in experiment 1 were 12.5 and 13.3, and in experiment 2 were 13.0 and 13.3 gm./100 ml., respectively.

The "k" can be derived by solving the equation. For example in treatment 4 of experiment 1 the computation will be:

$$I = k(Hb_2 - Hb_1) + M$$

$$0.4141 = k(15.7 - 12.5) + \frac{(4.78 - 2.65)}{49.11}$$

the "k" obtained is:

| | | | |
|---|---|---|---|
| Experiment 1. | treatment 4 | = | 0.1158 mM. |
| Experiment 1. | treatment 5 | = | 0.1558 mM. |
| Experiment 2. | treatment 5 | = | 0.1212 mM. |
| Experiment 2. | treatment 6 | = | 0.1275 mM. |
| | average | | 0.1301 mM. |

Thus every unit gm. of hemoglobin per 100 ml. of blood apparently was used to handle 0.1301 mM./lb. body weight of dietary $NO_2^-$ intake in these two experiments. The actual "k" value may not vary so much (0.1158 - 0.1558 mM.) but in performing the computations decimal numbers were rounded to make the rough estimation. Also, many variations in these experiments could not be explained as for example the fluctuation of methemoglobin level in the non-nitrite groups, differences between the blood samplings collected, etc. The population was small and we cannot expect all plus and minus deviations from the mean to cancel each other.

In both experiments nitrate did not have a marked effect on hemoglobin or methemoglobin levels of swine. There were no significant differences between control and nitrate nor between nitrate levels. This indicated that $NaNO_3$ is not converted into $NO_2^-$ in the gastrointestinal tract of swine under normal conditions. However, this conclusion may not be valid under certain conditions, for example when swine are fed a high roughage or high fiber ration along with nitrate salts of lower solubility. Under such conditions microorganisms in the lower digestive tract in the hog may convert $NO_3^-$ into $NO_2^-$ in the same manner as the rumen microorganisms.

Such a condition where $NO_3^-$ is converted into $NO_2^-$ in the gastrointestinal tract of swine should be comparatively rare. Wang, Cardia-Rivera and Burris (1961) found that a cow receiving 70-100 gm. $KNO_3$ daily showed no increase of methemoglobin in blood for several days.

Nitrate did not affect hemoglobin, methemoglobin or rate of vitamin A depletion in the trials reported herein. The ill effects of high levels of dietary $NaNO_3$ might be explained as being due to lowered feed intake and to metabolic interference of $Na^+$ on $K^+$ and $Cl^-$ in vivo, as was reported by Guylyassy, de Stribou and Schwartz (1962) who reported that dogs fed with only 11 mM. of $NaNO_3$ per kg. body weight daily had a negative balance of $K^+$ and $Cl^-$. Table 14 shows that the daily intake of $NaNO_3$ in experiment 2 ranged from 14.058 mM. to 30.564 mM. per kg. body weight, which was higher than the level of 11 mM. reported in dogs. It is suggested that the growth depressing effects of $NaNO_3$ in this experiment arose mainly or wholly from the interference of $Na^+$ on $K^+$ and $Cl^-$ metabolism. $Na^+$ is excreted mainly in the form of urinary NaCl and the Na:K ratio in urine always remains constant under normal conditions. Comparing experiment 1 to experiment 2, it is also suggested that the intake of 3.501 mM. or less of extra $Na^+$ per lb. body weight per day may not affect growth rate and feed consumption of growing finishing swine under normal conditions if the period is not too long.

In experiment 1, serum vitamin A was determined only twice, the first determination at the end of the experiment, the second after the pigs has been on pasture for 20 days. Pigs on this experiment had been on the pasture, with their mothers before the start of the experiment, thus they should have had liberal amounts of vitamin A stored in the liver.

Table 14. Dietary intake of $NaNO_3$ in the experiments.

| | Av. $NaNO_3$ per head daily, gm. | Av. $NaNO_3$ intake per lb. body weight daily mg. | mM. |
|---|---|---|---|
| Experiment 1 | | | |
| Treatment 2 (0.6-1.0% $NaNO_3$) | 18.2326 | 143.8469 | 1.6923 |
| Treatment 3 (1.2-2.0% $NaNO_3$) | 40.0246 | 1297.5867 | 3.5010 |
| Treatment 7 (0.6-1.0% $NaNO_3$) | 16.9614 | 142.5327 | 1.6769 |
| Experiment 2 | | | |
| Treatment 2 (3% $NaNO_3$) | 40.6012 | 543.1598 | 6.3901 |
| Treatment 3 (3% $NaNO_3$) | 50.6392 | 632.0024 | 7.4353 |
| Treatment 4 (5% $NaNO_3$) | 98.6020 | 1180.8622 | 13.8925 |

At the end of the experiment there were wide variation of serum vitamin A between treatments (Table 6). The drop of serum vitamin A in all treatments except the control indicated depletion of vitamin A in the body, both serum and liver vitamin A. There are several earlier reports showing that the serum vitamin A is related to liver vitamin A semi-logarithmically, especially when serum vitamin A drops down below the normal level of that species (Almquist, 1952; Braun, 1945).

The addition of $NaNO_3$ to the vitamin A deficient diet (ration 29A in treatment 6 and 7, experiment 1) did not give a significant difference in serum vitamin A at the end of the experiment. Values ranged from 6.04 to 8.01 mcg./100 ml. The serum vitamin A of pigs that were fed the normal 29D ration supplemented with $NaNO_3$ varied somewhat, ranging from 6.04 to 13.98 mcg./100 ml. However, none of them were lower than the pig fed on the vitamin A deficient ration (ration 29A). Serum vitamin A values of pigs fed the control ration supplemented with $NaNO_2$ fell far lower than all others ranging from 0.00 to 4.13 mcg. per 100 ml. of serum. Similar results were found in experiment 2 in which there were significant differences in serum vitamin A values between control and treatments, between nitrate and nitrite but not between nitrate levels (Table 11). It may then be concluded that supplemental $NaNO_3$ did not interfere with vitamin A destruction in the tissues as it did not accelerate the depletion of vitamin A as $NaNO_2$ did. It possibly did interfere with the utilization of carotene as a vitamin A source, either by interfering with carotene conversion or the absorption of vitamin A or both. This apparent interference did not increase as the level of dietary $NaNO_3$ increased. The difference between the effects of $NaNO_3$ and $NaNO_2$ also supports the conclusion that $NaNO_3$ was not converted into $NO_2^-$.

A tendency for lower serum vitamin levels when dietary $NO_2^-$ was increased was observed in both experiments. The Duroc seemed to be more depleted in serum vitamin A than the Duroc X Poland China crossbred, even when the Duroc was a gilt and had a slower growth rate than her mate on the same treatment. After 20 days of repletion on pasture, serum vitamin A was higher in the crossbred than in the Duroc, even though within the pair the crossbred had lower serum vitamin A at the end of the treatment. This result may indicate that the crossbred Duroc X Poland has a higher ability to utilize carotene as a vitamin A source. However, it is impossible to say whether this ability is due to heterosis, additive genetic effect of dominant genetic effect.

Pigs on both experiments showed no visible sign of a vitamin A deficiency even though one of them had a serum vitamin A value approaching 0.00 mcg./100 ml. This may have been because the pigs were not kept at such a low level of serum vitamin A long enough.

In the second experiment in which depleted pigs were fed ration 29D, which was computed to be rather high in vitamin A activity, the serum vitamin A at the start of the experiment varied markedly ranging from 6.8 to 29.8 mcg./100 ml. This was probably due to differences in both litters and growth rates of pigs. The heavier pigs tended to have lower serum vitamin A levels. After supplementation with nitrate and nitrite the serum vitamin A had a tendency to be reduced steadily in most of the pigs except those in the control group. The rise of serum vitamin A of the more depleted individuals on nitrate and nitrite treatments indicated that neither nitrate nor nitrite absolutely inhibits the conversion of carotene into vitamin A. When the weather was warmer, there was a rise in serum vitamin A in all treatments, as can be seen on

Table 12 in the blood samplings collected on February 26, 1963. This might possibly be because when the temperature rose above critical temperature the metabolic rate, or the catabolism of energy, dropped back to the normal rate and vitamin catabolism was then reduced accordingly. It was noted that serum vitamin A levels of some pigs rose to very high levels but the liver vitamin A was low. However, at the end of the experiment, liver vitamin A in mcg./gm. tended to be related to serum vitamin A in mcg./100 ml. A higher level of liver vitamin A in the females also was observed. The sex difference was not significant, but a significant interaction between sex and treatment was obtained in the analysis of variance for serum vitamin A value. That is, when the change in serum vitamin A was computed in percentage units, the serum vitamin A of the male increased at a higher rate under the favorable condition of liberal vitamin A intake and decreased at a faster rate under stress conditions (dietary nitrate or nitrite) interfering with vitamin A metabolism. The significant difference in hemoglobin between different carotene levels could have arisen from the continuous anemic tendency of a pig on a low carotene diet. There was no such difference in experiment 1.

The way (or ways) in which dietary nitrite affected serum and liver vitamin A levels cannot be explained from the results of this experiment. It was apparently masked by both sex and environmental temperature effects. The only conclusive statement which can be made is that $NO_2^-$ decreased body content of vitamin A. It may not be because $NO_2^-$ is an oxidizing agent which could oxidize vitamin A in blood serum because others have reported it is rapidly and tightly bound in heme part of methemoglobin in red blood cells (Wang, Garcia-Rivera and Burris, 1961). Nitrate or nitroso

might possibly be bound in the form of nitrosomethemoglobin as it is in nitrosomyoglobin in meat. Methemoglobin itself has been reported to be a poor catalyst for the oxidation of vitamin A in the tissues. It is possible that it caused increased destruction of vitamin A by causing an increase of hematin or related compounds released from the hemolysis of red blood cells, as reported by Dmitrovskij (1961).

The chemical analysis for nitrate and nitrite in blood and tissues indicated only a trace of nitrite in the blood taken from each of the pigs fed high nitrite, low nitrite, low nitrate and control. The determination showed that the concentrations were lower than 2 ppm. Nitrate and nitrite could not be detected in the rest of the blood samples and tissue extracts whereas it can be clearly detected even in normal human saliva. When saliva was put into the test tube containing tissue extract and reagents the pinkish red color was clearly obtained.

The calculated amount of methemoglobin retained in the body after the nitrite supplemented feed was withdrawn for a period of approximately 30 hours is shown in Table 15 and explained below.

Table 15 was obtained by applying the results of experiment 2, reported in the earlier paragraphs which indicated that 1 mM. of $NO_2^-$ per pound of body weight will increase methemoglobin level by 49.11% of total hemoglobin. The average value for methemoglobin of a normal animal was 2.57% which was the average for the blood samples collected from pigs not receiving nitrite. This was used as the standard to be subtracted out of the methemoglobin level of the three animals used for the computation of $NO_2^-$ and methemoglobin disappeared and retained in the body.

Table 15. Amount of methemoglobin retained and disappeared after a period of approximately 30 hours (Experiment 2).

| Treatment | Methb. % | $NO_2^-$/lb. weight/1 mM. | Methb. % after 30 hours | $NO_2^-$/lb. weight/1 mM. | Disappeared % |
|---|---|---|---|---|---|
| 5/2 (0.3%$NaNO_2$) | 10.27 | 0.15678 | 1.74 | 0.02383 | 84.8 |
| 6/2 (0.5%$NaNO_2$) | 29.47 | 0.54779 | 4.43 | 0.07865 | 85.7 |
| 6/2 (0.5%$NaNO_2$) | 28.23 | 0.52252 | 6.77 | 0.08777 | 83.4 |

/1 % expected to be in methemoglobin.
/2 Individual pigs.

It was observed that the rate of methemoglobin turn over was very rapid. About 84% of the methemoglobin disappeared in a period of approximately 30 hours after supplemental $NO_2^-$ was removed from the diet. Methemoglobin levels apparently did not affect the rate of disappearance of methemoglobin from the body. It then seems doubtful whether most of the $NO_2^-$ is handled mainly by methemoglobin. The concentration of nitrite ions in the body did not seem to be enough to do any harm to the public health in either experiment.

## SUMMARY

Results from the two trials may be summarized as follows:

1. It appeared that nitrate was not converted into nitrite in the gastrointestinal tract of swine and it did not seem to increase the rate of serum and liver vitamin A destruction. Nitrate appeared to interfere with the absorption or utilization of carotene as a vitamin A source. Nitrate had no significant effects on hemoglobin and methemoglobin levels in swine.

2. Dietary nitrite increased the rate of vitamin A depletion by increasing the destruction of vitamin A in the tissues.

3. Nitrite decreased hemoglobin level first and then increased methemoglobin level. It was suggested that the animal body had a mechanism to get rid of $NO_2^-$ at the expense of hemoglobin without the formation of methemoglobin. The pattern of ingested $NO_2^-$ in the body was suggested to be "$I = k(Hb_2 - Hb_1) + M$".

4. Rate of methemoglobin turn over was found to be about 84% within a period of approximately 30 hours after supplemental $NO_2^-$ was removed from the diet. The rate of turn over was found to be independent of the amount

of methemoglobin retained in the body.

5. Growth depressing effect of sodium nitrite was suggested to be primarily due to the reduction of feed consumption; of sodium nitrate by the interference of excessive $Na^+$ on $K^+$ and $Cl^-$ metabolism. Another factor was reduced feed consumption.

6. The crossbred (Duroc X Poland) seemed to have better ability to utilize carotene as a vitamin A source than the purebred Duroc.

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# SOME EFFECTS OF DIETARY NITRATE AND NITRITE ON METHEMOGLOBIN LEVEL, CAROTENE CONVERSION, WEIGHT GAIN AND FEED EFFICIENCY IN GROWING SWINE

by

SIRIPONG SUKHONTHASARNPA

B. S., Kasetsart University, 1959

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ABSTRACT

Two experiments designed to study the effect of dietary nitrate and nitrite on growing finishing swine have been completed. In the first experiment, (summer) 0.6-2.0% $NaNO_3$ and 0.15-0.4% $NaNO_2$ added to the ration did not have any significant effect on growth rate, feed consumption or feed conversion efficiency. In the second experiment, (winter) the supplementation of 3 or 5% $NaNO_3$ and 0.3 or 0.5% $NaNO_2$ significantly reduced growth rate ($P<.05$). The growth retarding effect of $NaNO_2$ was suggested to be due to the lowered daily feed consumption.

$NaNO_3$ was not converted into $NO_2^-$ under conditions of these experiments and apparently interfered only with the conversion of carotene to vitamin A in the intestine or interfered with the absorption of vitamin A. $NO_2^-$ apparently increases the rate of vitamin A destruction in the body whereas $NO_3^-$ does not. Supplementary dietary $NaNO_2$ reduced hemoglobin levels in both experiments significantly. It was suggested that the animal body has a mechanism to get rid of $NO_2^-$ in vivo at the expense of hemoglobin. This mechanism is a primary one and is limited by the level of hemoglobin the animal has to maintain. After this mechanism is exhausted, the methemoglobin will increase additively as $NO_2^-$ in the body is increased. The hemoglobin level swine have to maintain was found to be 12.5-13.3 gm./100 ml. and is independent of the amount of hemoglobin originally in the body. Every 1 mM. of $NO_2^-$ ingested per pound of body weight is capable of increasing methemoglobin level to 48.7-49.1% of total hemoglobin. The pattern of $NO_2^-$ in vivo was suggested to follow the model "$I = k.(Hb_2 - Hb_1) + M$" when I is mM. of $NO_2^-$ intake per pound body weight, k is mM. of $NO_2$ which can be handled by 1 unit gm./100 ml. of

hemoglobin, ($Hb_2 - Hb_1$) is the level of hemoglobin that an animal can use to handle $NO_2^-$ without the formation of methemoglobin, M is mM. of $NO_2^-$ which will be bound in methemoglobin. k was found to be 0.1301 mM. in this experiment.

The rate of methemoglobin turn over seems to be very rapid. About 84% of the methemoglobin disappeared in a period of approximately 30 hours after supplemented amounts of $NO_2^-$ were removed from the diet. Turn over rate was apparently independent of the original level of methemoglobin in the body. Toxic effects of $NaNO_3$, when ingested at high levels, were suggested to be due to the interference of $Na^+$ on $K^+$ and $Cl^-$ metabolism.