THE EFFECT OF HYPERVITAMINOSIS A AND OTHER DIETARY FACTORS ON THE YOUNG PIG

by

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INTRODUCTION

Research on vitamin A and its various ramifications has proceeded for nearly half a century, yet a complete understanding of the role this vitamin plays in body metabolism still eludes investigators. Relatively great progress has been made in the past 10 years, however, in elucidating the specific bodily processes requiring vitamin A for normal function and in determining some of the biochemical steps in which it is involved.

A renewed interest in vitamin A for livestock feeding has taken place in recent years as producers have become aware that more satisfactory feeding performance could often be obtained by increasing the quantity of vitamin A available to the animal in amounts greater than earlier established requirements and recommendations. With greater awareness of the need for vitamin A in livestock feeding have come reports suggesting and inquiries concerning vitamin A toxicity. The question of carotene toxicity has also been raised. The voluminous literature on vitamin A apparently does not contain references to hypervitaminosis A in swine.

The work reported herein was conducted to determine some of the characteristics of hypervitaminosis A in the young pig and to determine if certain other dietary factors may influence this condition.

REVIEW OF LITERATURE

Symptoms of Toxicity and Effects of a High Level of Vitamin A

Following reports of arctic explorers who became ill after the ingestion of polar bear liver, Rodahl and Moore (1943) analyzed samples of seal and polar bear liver for vitamin A and found them to contain from 13,000 to 18,000 International Units (IU) of the vitamin per gram of wet weight. When rats were given approximately one gram of this liver daily, they became anemic, were paralyzed in the rear legs, and hemorrhaged under the skin and in the pericardium. Evidence for a malfunctioning blood-clotting mechanism was found. Not all animals were affected, however. These characteristics are typical of most reports on vitamin A toxicity.

Maddock et al. (1948) found high levels of vitamin A fed to rats resulted in hemorrhages in the subdural space, epididymus, skeletal muscles, and in the body cavities. The lymph nodes were hemorrhagic in appearance because their sinuses were filled with blood. Hematocrit values fell to 13% and prothrombin times were three to eight times greater than those of control animals. Light et al. (1944) had shown that hypoprothrombinemia due to overdosages of vitamin A alcohol or ester could be corrected by daily administration of vitamin K. Dowling (1961) found that vitamin A acid in

excess would cause bleeding about the eyes and nose as well as loss of hair. Rodahl (1949) found in detailed studies of prolonged administration of vitamin A excess to rats, mice, guinea pigs, rabbits, dogs, and chickens that symptoms resembling those of scurvy often developed. In addition, microscopic examination of teeth indicated disarrangements of the odontoblasts and amorphous calcification of the inner dentine as well as deposits of calcium in the pulp.

Rodahl (1950) observed the following effects due to hypervitaminosis A in the rat: pelt deterioration, muscular weakness, lack of appetite, reduced growth, soreness and bleeding of skin, alopecia, swelling of palpebrae, exophthalmos, stiffness of limbs, spontaneous fractures, hemorrhages, proteinuria, hematuria, diarrhea, intestinal bleeding, and anemia. No significant changes were found in blood calcium, phosphorus, potassium, total base, urea, or sugar. Bone ash was reduced, but bone ash calcium or phosphorus was not affected. Histologically, the usual findings were renal tubule degeneration, deposits of sudanophil droplets in the liver and in the adrenal cortex as well as general internal hemorrhage.

Hypervitaminosis A in the dog, which is apparently more resistant to vitamin A toxicity than rats and mice, was examined by Maddock et al. (1949). The results were similar to those previously described above with rats, but in addition

it was found that serum cholesterol increased as did lipoid phosphorus in the blood. Some liver necrosis was found and vascular lesions in the media of arteries and veins, myocardium, gall bladder, and urinary bladder were observed. Wheeler (1945) reported that diets high in vitamin A or vitamin A precursors caused diarrhea in dogs. Maddock and Wolbach (1950) and Nerurkar and Sahasrabudhe (1956) found high levels of vitamin A to cause negative nitrogen, calcium and phosphorus balances in rats. Dull et al. (1961) found that 200,000 IU per day to adult humans markedly depressed serum and urinary citrate and decreased serum calcium, but that serum phosphorus and alkaline phosphatase were unchanged. Proll and Ketz (1963) with rats, however, found that 30,000 IU of vitamin A intramuscularly daily for 10 days had no effect on blood or urine citrate concentrations.

Chicks given 100,000 IU of vitamin A per day intramuscularly, but not those given 50,000 units daily, showed weight gain depression, increased mortality, and increased feed per pound gain according to Squibb (1963). The higher level of vitamin A caused an increase in liver lipids and a decrease in liver protein compared to control chicks. The liver, however, seems to be able to store tremendous amounts of vitamin A with few adverse effects (Davies and Moore, 1934, 1935; Rodahl and Moore, 1943).

Kupffer cells of the liver apparently store excesses of

vitamin A, but "true" liver storage of the vitamin occurs in the liver cells proper (Popper and Brenner, 1942). Berdjis (1963) reported that excess vitamin A in rats resulted in increased size and number of mast cells, especially in the lymph nodes, gastrointestinal submucosa, and subcutaneous tissues.

Masek and Hruba (1962) found when toxicity symptoms appeared in rats after 50 days on a diet providing 45,000 IU per day that the adrenal gland weight had increased, but vitamin C content of the adrenals had decreased compared to that found in control animals. Liver fat content increased as a result of the high level of vitamin A also.

Hypervitaminosis A in a young child resulted in hepatomegaly, increased serum lipids and alkaline phosphatase, and localized periosteal swelling (Toomey and Morissette, 1947). Josephs (1944) had observed increased serum lipids and cholesterol content and lowered basal metabolism when children consumed excessive amounts of carotenoids and questioned whether carotenemia is harmless in the human.

March and Biely (1963) found that there was a mutual interference between vitamin A and cholesterol during the course of absorption across the intestinal walls of chicks. Large amounts of vitamin A given orally reduced the increase in serum cholesterol due to one percent added cholesterol in the diet.

Doses of vitamin A to rats in quantities great enough to

cause death in two or three weeks produced no alterations in the reproductive tract or in the reproductive cycle according to Poumeau-Delille (1943). On the other hand, however, Sherwood et al. (1936) and Sherwood et al. (1937) found that either subcutaneous or oral administration of carotene in large amounts disrupted normal estrus cycles in rats by stimulating growth of the vaginal epithelium as indicated by the large number of nucleated epithelial cells in vaginal smears.

It appeared to Sherman (1961) that deficiency and excess of vitamin A both depress the rate of mitosis in certain epithelial tissues. Weiss and James (1955), using tissue culture techniques, found that exposure of embryonic chick epidermis to a media containing 0.06% vitamin A acetate for 15 minutes caused metaplasia from squamous, keratinizing epithelium into a cuboidal, mucus-producing tissue. Much the same results were found earlier by Fell and Mellanby (1953) for when embryonic chick ectoderm was cultivated in vitro in a medium containing excess vitamin A, keratinization was completely suppressed and, instead, a differentiation into mucus-secreting, often ciliated epithelia resulted.

These results imply that vitamin A may play a role in cellular and tissue differentiation and may help to explain the reason congenital malformations were observed in mice (Walker and Crain, 1960; Kalter and Warkany, 1961) and rats (Berdjis, 1958; Cohlan, 1953) when their dams were given high

levels of vitamin A during pregnancy. Vitamin A deficiency in pregnant rats also results in deformed young (Wilson et al., 1953). Adult pregnant rats given 25,000 to 50,000 IU of vitamin A per day by Moore and Wang (1943) developed fatal uterine hemorrhages or subpericardial hemorrhages. Subcutaneous and intramuscular hemorrhages were also found. These authors also remarked that there seemed to be a superficial resemblance to vitamin C deficiency in these rats. The adult rats, in contrast to young growing ones, did not suffer any bone fractures, however.

Vitamin A deficiency in pigs increases spinal fluid pressure (Frape, 1957), but so does hypervitaminosis A in rats (Becker and Sutton, 1963). The latter authors concluded that the increased spinal fluid pressure was caused by increased vascular to ventrical protein transport in the choroidal epithelium.

Sadhu and Truscott (1948) found that hypervitaminosis A caused a decrease in thyroid weight and in the amount of protein-bound iodine in the thyroid and in the liver, but an increase in the protein-bound iodine in the serum, pituitary gland and skeletal muscle. They concluded that these effects were due to decreased hepatic destruction of thyroxine due to the high levels of vitamin A. Later Ray and Sadhu (1959) found hypervitaminosis A to inhibit liver respiration by affecting dehydrogenase activity or an immediate step follow-

ing it, but the cytochrome c-cytochrome oxidase end of the succinoxidase system is little affected. Sampson et al. (1962), however, concluded on the basis of a study of oxygen consumption by rats given excess vitamin A that the thyroid gland was hyperactive during the later stages of vitamin A poisoning.

Vitamin A acid will produce toxicity symptoms in rats more quickly and at a lower dosage than vitamin A alcohol (Thompson and Pitt, 1960; Thompson and Pitt, 1961). Thompson et al. (1961) used sublethal doses of vitamin A acid to show that male rats so treated lost their ability to produce sperm.

Wolbach (1946) and Wolbach and Hegsted (1952) have developed the theory that excessive vitamin A accelerates the normal growth sequences of bone, especially as related to epiphyseal cartilage cell cytomorphysis, cells which they consider to control the pattern of bone growth. Van Metre (1947) published work supporting this theory. A different view is taken by Irving (1949), however, who considered the effect of hypervitaminosis A on bone to be one of depressed osteoblastic activity. The osteoclasts appear to be unaffected with the result being that bone so affected becomes abnormally thin and subject to fractures. He also found that less dentin was produced by odontoblasts under the influence of high levels of vitamin A.

Rat femur weight and breaking strength were decreased in

rats given 24,000 IU per day (Khogali, 1960). Calcium retention was decreased, but tibia percent ash was unaffected although bones were reduced in thickness. Khogali questioned whether the effect on bone was due to remodeling processes or to an effect due to calcium availability.

Rabbit articular and costal cartilage chondrocytes lost their ability to take up sulfate ($S^{35}O_4$) due to vitamin A toxicity, and when S^{35} was incorporated into bones of live animals and then the animal given a massive dose of vitamin A intraperitoneally, the S^{35} was rapidly released from these tissues (McElligott, 1962). Cartilage cells were depressed and sulfur was lost from cartilage matrix of rabbits given large amounts of vitamin A (Thomas et al., 1960). Frape (1957), starting with vitamin A deficient pigs, found that the specific activity of S^{35} in costochondral junctions and other tissues decreased with increasing levels of dietary vitamin A except at his highest level (11393 IU/lb. of diet) where the specific activity increased. With rats, Dziewiatkowski (1954), on the other hand, found that administration of vitamin A to vitamin A deficient animals increased the uptake of sulfur and phosphorus by the bone, especially near the epiphyseal cartilage plate. Fell et al. (1956) concurred with these results.

Hypervitaminosis A causes an increase in alkaline phosphatase in the epiphyseal junctions of certain bones, but

others seem to show reduced contents of phosphatase (Ludwig, 1953).

Closure of the epiphyseal junctions of tibial and femoral bones of rachitic rats was brought about rapidly with massive doses of vitamin A even though normally these junctions remain open during most of the rat's life (Wolbach and Maddock, 1949).

According to Wolbach and Hegsted (1952), the histological sequence of the normal pattern of bone growth was accelerated in young chicks with the administration of high levels of vitamin A, but Rigdon et al. (1951) found no pathologic or roentgenographic evidence that the bones of young ducks given up to 200,000 IU of vitamin A per day were affected. Bone growth in birds is not the same as bone growth in mammals in all respects, however.

Berdjis (1959) compared hyperparathyroidism with hypervitaminosis A on bone and concluded that both can cause resorption of bone with subsequent lacunar fibroosteoclasia, but only hypervitaminosis A affected cartilage to any extent. He considered the slight hyperplasia of the parathyroids with high levels of vitamin A as a result of bone changes rather than the cause of them. Wolbach and Maddock (1952) believe that the effect of high levels of vitamin A is not mediated through the pituitary or the adrenal glands.

Uhr et al. (1963) found with guinea pigs that the

clearance of injected bacteriophage from the circulation was not affected by vitamin A at high levels nor was antibody formation to the phage affected.

Gerriets (1961) found that increasing the vitamin A content of the diets of chicks from 500 to 5,000 IU per pound reduced mortality from cecal coccidiosis by 90%, but Squibb and Veros (1961) found that doses of up to 50,000 IU to partially depleted chicks had no effect on the intensity or course of infection from experimentally induced Newcastle disease virus. Biely et al. (1962) found that 44,000 IU of vitamin A per pound of feed had no effect on egg production, but 220,000 and 440,000 IU markedly depressed it.

Lactating cows showed no gross effect to 1,250,000 IU of vitamin A fed daily for three months (Wise et al., 1947). Total milk and milk fat, as well as milk fat percentage, were not altered, but the vitamin A content of the milk increased somewhat while the carotene content decreased.

Vitamin A Excess in vitro

Fell (1960, 1964) reviewed the in vitro work her group had conducted in an attempt to study the mode of action of vitamin A on bone by using tissue culture media high in vitamin A concentration. When the tissue culture media had a concentration of vitamin A similar to the concentration found in the blood of animals suffering from hypervitaminosis A,

Fell and Mellanby (1951, 1952) found that there was a retardation of chondroblastic hypertrophy and ossification. Furthermore, the cartilage matrix of embryonic limb-bones in this medium shrank and lost its basophilia and metachromasia; however, these changes were not associated with cell degeneration. Similar results were found with media to which papain was added (Fell and Thomas, 1960). Neither papain or a high level of vitamin A had much effect on existing bone in general, but there was an additive effect of vitamin A and papain on cartilage. The wet weight, dry weight, and amino sugar content of the bone rudiments decreased as did ribonucleic and desoxyribonucleic acid phosphorus of the chick-limb bone cartilage subjected to a media high in vitamin A concentration (Fell et al., 1961). Hydrocortisone depressed these effects of excess vitamin A in tissue culture (Fell and Thomas, 1961) in contrast to that in the intact animal where the effects seem to be additive (Weissmann, 1961).

In 1959, de Duve summarized his work which had resulted in the isolation and description of previously unknown subcellular particles which contain a variety of enzymes or enzyme precursors having an acid pH optimum and which he called lysosomes. Fell and her associates began to consider that the mode of action of excess vitamin A (and perhaps normal, physiological amounts of vitamin A) could possible be related to the structure or state of these cellular particles

(lysosomes). In a series of papers, the effect of high levels of vitamin A on the release of enzymes from the lysosomes and the resulting effect on the tissue was explored (Dingle et al., 1961; Dingle and Lucy, 1961; Lucy et al., 1961; Dingle, 1961; Fell et al., 1962; Fell and Dingle, 1963). It was concluded that cartilage matrix breakdown in response to a high concentration of vitamin A was due to an acid protease released from cell lysosomes. This was responsible for loss of cartilage metachromatic material. Weissmann et al. (1963b) found some of the same effects of hypervitaminosis A on lysosomes in the intact guinea pig as Fell's group had in in vitro studies. Chrondrocytes are not prevented from synthesizing hexosamine, but in the presence of high concentrations of vitamin A, the hexosamine is liberated into the surrounding media (Fell, 1964).

The detrimental effects on lysosomes in in vitro techniques seems to be relatively specific for high levels of vitamin A, at least as tested (Fell et al., 1962); however, Zalkin et al. (1961) and Zalkin et al. (1962) believe that muscle lysosomes are caused to release their enzymes (cathepsin, ribonuclease, beta galactosidase, aryl sulfatase, etc.) in muscular dystrophy of vitamin E deficient rabbits and that this is due to lipid peroxidation of the lysosomal membrane. Morrison et al. (1963) found that potassium-deficient diets would cause lysosomes to become prominent in renal papillae

of rats when examined by light and electron microscopy. Acid phosphatase (presumably from the lysosomes) also was increased in concentration in these tissues at this time.

Vitamin A in excess caused mitochondria, both isolated and intracellularly, to become swollen with their internal structure being distorted, an effect similar to that which was also found with thyroxine (Lucy et al., 1963; Dingle et al., 1962). That due to vitamin A excess could be prevented by alpha-tocopherol (Lucy et al., 1963).

Vitamin A in excess causes local distention and/or disintegration of the cell membranes of erythrocytes and fibroblasts (Dingle et al., 1962). Glauert et al. (1963) and Dingle (1964) reported bizzare shapes of rabbit erythrocytes and rapid hemolysis were results of high levels of vitamin A. Dingle and Lucy (1963) found that membrane instability produced by excess vitamin A is largely inhibited by equimolar quantities of vitamin E. As a result of largely in vitro work with erythrocytes in a medium of a high concentration of vitamin A, it has been suggested that one of the physiological roles of vitamin A is to stabilize membranes by acting as a cross-linking agent between lipid and protein molecules (Lucy and Dingle, 1962; Dingle and Lucy, 1962). Blaugh (1963) has data to support this concept.

Vitamin A Activity as Related to Other Substances

Vedder and Rosenberg (1938) used the most potent source of vitamin A known at the time, liver oil from the jewfish (Stereolepis gigas), to produce vitamin A toxicity in rats and found that vitamin D at dosages less than that of vitamin A decreased the incidence of death and bone fractures due to the high level of vitamin A. When the dosage of vitamin D exceeded that of vitamin A, the combination was more toxic than that of the vitamin A alone. They suggested that the toxic effects of jewfish oil at high levels was not due to its vitamin A content alone, however. Moore and Wang (1945) used pure crystalline vitamin A to produce toxicity in rats and found that one milligram of calciferol per day had no suppressive action on the incidence of bone fractures or hemorrhages resulting from the vitamin A.

Adding carrots as a source of provitamin A to a diet for rachitic rats, together with zero or small amounts of vitamin D, had no effect on the rachitic state as indicated by the resulting bone ash composition. At a higher level of vitamin D, however, the carrot addition to the diet seemed to enhance the antirachitic effect of vitamin D (Bacharach et al., 1931).

Wolbach and Maddock (1949) found that massive doses of vitamin A would repair rachitic lesions in rats including resumption of normal epiphyseal cartilage cell cytomorphosis

and calcification of the cartilage matrix and osteoid. Surprisingly, under this treatment, epiphyseal closure took place although normally these remain open for most of the lifetime of the rat. Maddock et al. (1948) reported that massive doses of vitamin A to rachitic rats produced typical hemorrhagic lesions. Clark and Bassett (1962) found that vitamin A (30,000 IU) administered with a toxic dose of vitamin D (60,000 IU) in rats prolonged life, reduced the severity of kidney tissue calcification, and prevented calcification of degenerating muscle fibers and of the intima of large blood vessels compared to the effects of this level of vitamin D alone.

Skaloud (1947) found both vitamins D and C to show some compensating action on the effects of excess vitamin A on the bones and teeth of rats.

Large doses of cod liver oil inhibited the antiscorbutic action of vitamin C in guinea pigs, but 2,400 IU of vitamin A per day and a high level of vitamin D seemed to reinforce the action of dietary vitamin C (Collett and Eriksen, 1938). They concluded that there was no antagonistic action on vitamin C by a high level of vitamins A or D in guinea pigs. Vedder and Rosenberg (1938) completely prevented the toxic effects of 100,000 IU of vitamin A from jewfish oil by administering 5 mg of ascorbic acid to rats. With toxicity due to a crystalline vitamin A source, Moore and Wang (1945) found some of

their rats to exhibit symptoms resembling those of scurvy described in other species. They were unable to prevent bone fractures and hemorrhaging in the rats by administering 10 mg of ascorbic acid daily, however. Liver, adrenal, and urine content of ascorbic acid was not significantly affected by the vitamin A toxicity.

Rodahl (1949) also reported the similarities of some aspects of vitamin A toxicity and scurvy in the long bones of rats which they had examined microscopically. In most of these animals, there was abnormally low ascorbic acid concentration in serum, adrenals, and liver, but ascorbic acid deficiency was not considered to be the sole causative factor of vitamin A toxicity. High doses of vitamin A to rats were also found to decrease liver ascorbic acid content by Morehouse et al. (1952), but supplementary ascorbic acid had no beneficial effect in either preventing hypervitaminosis A or in raising the ascorbic acid content of the liver.

Wendt and Schroder (1935) found that vitamin C fed to guinea pigs with a high level of a fish liver oil source of vitamin A prevented the appearance of significant vitamin A toxicity symptoms and markedly suppressed the increased liver stores of vitamin A which occurred in those animals fed no vitamin C. When guinea pigs were given toxic doses of a purified source of vitamin A, however, there was no indication of an ameliorative effect of vitamin C on the vitamin A

toxicity symptoms (Simic et al., 1953).

Walker et al. (1947) found that massive doses of vitamin C to rats did not prevent bone fractures or hemorrhages due to hypervitaminosis A, but vitamin K diminished the incidence of hemorrhage although it had no effect on bone fractures. Vitamin K was partially effective in increasing hematocrit values which were depressed by hypervitaminosis A.

High levels of vitamin A, as either acid or ester, had similar effects on producing hypoprothrombinemia in rats which could be prevented by administering vitamin K, but 40,000 units of carotene per day did not affect blood clotting time (Light et al., 1944). High levels of vitamin A acid were found to be more hemorrhagic than those of vitamin A acetate by Matschiner and Doisy (1962). Quick and Stefanini (1948) concluded that the hypoprothrombic effects of high levels of vitamin A in chicks was due to an effect on the synthesis of vitamin K by the intestinal microflora.

Hemorrhage in developing amphibia suffering from hypervitaminosis A was prevented by administration of epsilon-amino-caproic acid by Weissmann et al. (1963a).

Small amounts of tocopherols seem to increase the efficiency of conversion of carotene to vitamin A, but large amounts interfere with this activity (Herbert and Morgan, 1953; Johnson and Baumann, 1948; Harris et al., 1944). Large amounts of synthetic antioxidants act similarly to large

amounts of tocopherols (High et al., 1954) possibly by interfering with the oxidative steps involved in the conversion of carotene to vitamin A.

Johnson and Baumann (1948) found that 5-10 mg of alpha-tocopherol per day did not interfere with the storage or utilization of vitamin A in contrast to its effect on carotene, however, Hickman et al. (1944) reported that utilization of vitamin A, as such, for growth was depressed by high levels of mixed tocopherols.

Weitzel et al. (1956) fed high levels of vitamins A, E, and K alone and in combinations to old, atherosclerotic hens, and found that vitamin A (7-10 mg per day) reduced the incidence of atheromatosis in all test groups as well as decreased the total fat content of the aortas. Vitamins A and E (50 mg per day) acted synergistically in decreasing the number of fat plaques and total fat and cholesterol content of the aortas. Wood and Topliff (1961) considered that the vitamin A content of fish oil was responsible for up to 85% of its activity in preventing the three-fold increase in serum cholesterol due to one percent added cholesterol in the diet of chicks.

Rats receiving 30,000 IU of vitamin A per day had depressed basal metabolic rates (BMR) and reduced thyroid gland weights according to Sadhu and Brody (1947). Veil et al. (1961) also found that excess vitamin A to rats in their study

lowered their BMR, but could find no significant effect on thyroid weight or on the histology of the gland. That massive doses of vitamin A provoked no histological effect on the thyroid of rats was also reported by Poumeau-Delille (1943).

Injections of thyroxine to rats receiving excess vitamin A did not counteract the effect of hypervitaminosis A and, in fact, had an additive effect with decreased feed consumption and survival time and in increased weight loss (Bauman and Moore, 1939). Excess vitamin A did not affect the fixation of iodine by the thyroid or the distribution of iodine in the gland according to Veil et al. (1961), but Lucjuk (1961) reported that his experiments indicated that high levels of the vitamin reduced the iodine content of the gland and increased its weight. Methyl thiouracil increased these effects when administered at the same time. Veil et al. (1961) found that labeled thyroxine injected peritoneally was excreted more rapidly by those rats given excess vitamin A than those receiving lesser amounts. Also, resulting specific activities of liver and kidney tissue were greater in the excess A group than in the control animals.

Excess vitamin A acid induced into the larva of an amphibian undergoing metamorphosis induced gross malformations in developing tissue, but these could be largely prevented by simultaneous administration of hydrocortisone (Weissmann et al., 1963a). Simultaneous administration of hydrocortisone

with a toxic amount of vitamin A alcohol, however, accelerated the rate of malformations (Weissmann, 1961). Thomas et al. (1963) found that hypervitaminosis A in rabbits produced by oral administration of either the ester or the acid form of the vitamin caused collapse of the distal third of the ears due to cartilage matrix depletion. Simultaneous administration of cortisone largely prevented this effect. According to Wolbach et al. (1955) cortisone has no effect in preventing skeletal deformities in rats due to vitamin A toxicity, but adrenalectomy accelerates this action of the vitamin.

Millen and Woollam (1957) found cortisone to increase the incidence of malformed fetuses due to administration of high levels of vitamin A to rats during pregnancy. Selye (1957) claimed that somatotrophic hormone in his experiments had prevented any effect on the bones of rats due to 20,000-30,000 IU of vitamin A per day compared to control animals not receiving the hormone. Administration of growth hormone to pregnant rats by Cohland and Stone (1961) did not alter the teratogenic effect of vitamin A excess and no support for the claim that cortisone potentiates the action of a high level of vitamin A could be found. It appeared to these investigators that neither the adrenal, parathyroids, nor thymus glands were involved in vitamin A toxicity.

Ershoff et al. (1957) found that massive, but relatively non-toxic, doses of vitamin A to rats were potentiated to

produce significant symptoms of hypervitaminosis A by supplementation with alfalfa meal and succulent plants. Dried alfalfa juice and the water washed alfalfa pulp were also active in this regard. Supplementation of all known nutrients had no significant potentiating effect. Further studies on the water soluble extract of alfalfa failed to identify the potentiating substance (Ershoff and Hernandez, 1960).

Fitch (1943) and Fitch and Ewer (1944) reported on observations and experiments of "true" rickets occurring in sheep grazing green winter pastures in Australia and New Zealand. Further studies by Ewer (1948, 1949, 1950) revealed that weekly administration of cod-liver oil to the sheep decreased the incidence of rickets, but weekly drenching with a bone-flour slurry had no prophlactic effect. It was suggested that something in the green feed was interfering with phosphorus uptake and metabolism. Artificially dried green hay also produced the symptoms, not only in sheep, but also in guinea pigs. Samples of green oat hay were artificially dried and assayed for vitamin D. It was estimated that the sheep which exhibited the rachitic symptoms when fed the artificially dried hay were receiving five times their estimated requirements for vitamin D from the hay.

Grant (1951) and Weits (1952) studied the problem and suggested that vitamin D was not being absorbed by the animal in adequate amounts. Later, Grant (1953) suggested that high

levels of carotene in the green feed was causing the rickets, and after analysis of the forage, calculated that sheep could ingest 1-2 million IU of vitamin A as carotene per day and cattle 10-20 million units daily from the green feed.

Ewer (1953), Weits (1954), and Grant and O'Hara (1957) began to isolate chemically the substance in green feed causing the rickets, and it was concluded by the last authors that carotene was the causative factor. Weits (1960) concurred in these results and also found that the harmful effects of vitamin A excess (carotene excess) became manifest when the animals were on the borderline of vitamin D deficiency.

Lewis (1954) encountered conditions in pigs which resembled rickets, but which on further investigation proved to be due to hypervitaminosis A. The condition developed in pigs approximately eight weeks of age and weighing 60 to 80 pounds after consuming for two to three weeks a diet containing 67,800 IU of vitamin A per pound.

INVESTIGATIONS

General Procedures

The experiments reported herein are on file in the Swine Nutrition Section of the Animal Science Department, Iowa State University, Ames, Iowa. These are numbered as Swine Nutrition Experiments 1179, 6311, 6318, 6327, 6404, 6409, 6421, and 6428.

All animals used in these experiments were obtained from the swine nutrition farm breeding herd and were of cross-bred breeding. Within 24 hours after birth, each pig was individually weighed and ear notched, eye teeth were clipped, and 100 milligrams of iron, as iron-dextran, was injected intraperitoneally. Male pigs were castrated at approximately one week of age.

In all experiments the pigs were self-fed and had access to an automatic or continuous-flow waterer at all times.

Animals which died during the experiments were immediately weighed as were their pen mates so that feed consumption by the pig removed and those remaining on experiment could be estimated. Dead pigs were sent to the Veterinary Diagnostic Laboratory for examination or were examined and then used as sources of tissue for histological or biochemical analysis. Statistical analysis of the data collected was carried out according to the methods of Snedecor (1956).

Composition of the basal rations used in the experiments is given in Table 4 and calculated analysis of these rations is found in Table 5 in the Appendix.

Experimental

Experiment 1179 - Vitamin A studies with swine

Objectives The purpose of this experiment was to study the effect of increasing levels of dietary vitamin A on growth, feed conversion, and certain biological measurements as well as to determine symptoms of hypervitaminosis A in swine.

Experimental Thirty pigs averaging 18.6 pounds and 35.1 days of age were allotted to one of five ration treatments. The dietary treatments were 0, 500, 5,000, 50,000, and 500,000 IU of added vitamin A palmitate per pound of diet. Feed and water were provided ad libitum.

The experiment was conducted in unit A at the swine nutrition farm which provided 10 concrete-floored pens, each equipped with a self-feeder and continuous-flow waterer. The pens were cleaned daily and bedding, in the form of wood shavings, was provided. Heat lamps were used in each pen and the room temperature was maintained at approximately 70°F. This experiment was conducted during December, 1962 and January, 1963.

There were three pigs per pen and two replications of each treatment. Pigs were weighed and feed consumption determined and recorded weekly. The experiment was terminated after four weeks, at which time blood samples were drawn from the vena cava following an 18-hour fast, and metatarsal bones removed for analysis by the method of Zimmerman (1960).

Results and discussion After two weeks on the experimental diets, several pigs on the diet containing the highest level of vitamin A, 500,000 IU per pound of feed, began to show symptoms of hypervitaminosis A. The first death loss occurred at this time, however, the pig which died was healthy in outward appearance, although slightly smaller in size than his pen mates. Within a week, all the other pigs on this treatment exhibited symptoms of hypervitaminosis A of various degrees. The larger pigs were apparently more resistant than the smaller ones, and of pigs of the same weight, the slower growing ones usually were affected first.

The first obvious indications that the pigs were being affected by the high level of vitamin A, other than sudden death, were a general unthrifty appearance, and a reluctance to move about the pen. Shortly thereafter some of the animals lost their ability to use their rear legs. Later, strength and control of the front limbs was also greatly diminished. Growth ceased, and generally weight losses were incurred. There was considerable lacrimation.

Joints became swollen, especially in the rear legs. Hair coats were ragged in appearance and the skin, especially over the legs and abdomen, became dry and scaly. The skin around the top and between the hooves was cracked, and in some instances, bleeding occurred from these sites. Marked petechial hemorrhages appeared subcutaneously over the limbs and abdomen. Frequently the urine and feces contained blood.

Postmortem examination of the three pigs which died revealed that hemorrhages into several limb joints had occurred. In the intestine, subserosal and mucosal hemorrhages were evident. Subpericardial hemorrhage was found. Costochondral junctions were enlarged and, when cross-sectioned, the metaphyseal region was white and had the appearance of being decalcified. Histologically, the cartilage columns were disorganized. In one case a ruptured kidney with resulting hemorrhage into the peritoneal cavity was observed. Hemorrhages were noted in the area of the cortico-medullary junction of both kidneys of this animal.

Pigs other than those receiving 500,000 IU per pound of diet exhibited no symptoms of hypervitaminosis A at any time.

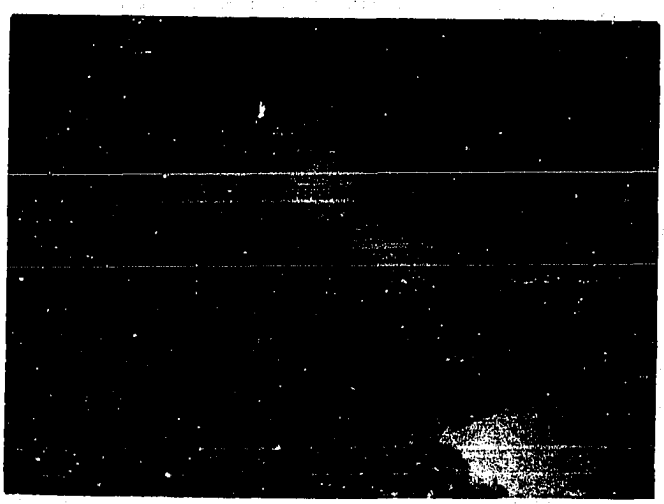
When metatarsal bones were removed for analysis, it was evident that the blood clotting mechanism in those pigs on the highest level of vitamin A was defective.

Photographs of some of the pigs are presented on page 29 in Figures 1, 2, and 3. Figure 1 is an example of the more

Figure 1. Experiment 1179 - Pig after 4 weeks on a diet containing 500,000 IU of added vitamin A per pound

Figure 2. Experiment 1179 - Same treatment as in Figure 1

Figure 3. Experiment 1179 - Pigs after 4 weeks on a diet containing 5,000 IU of added vitamin A per pound

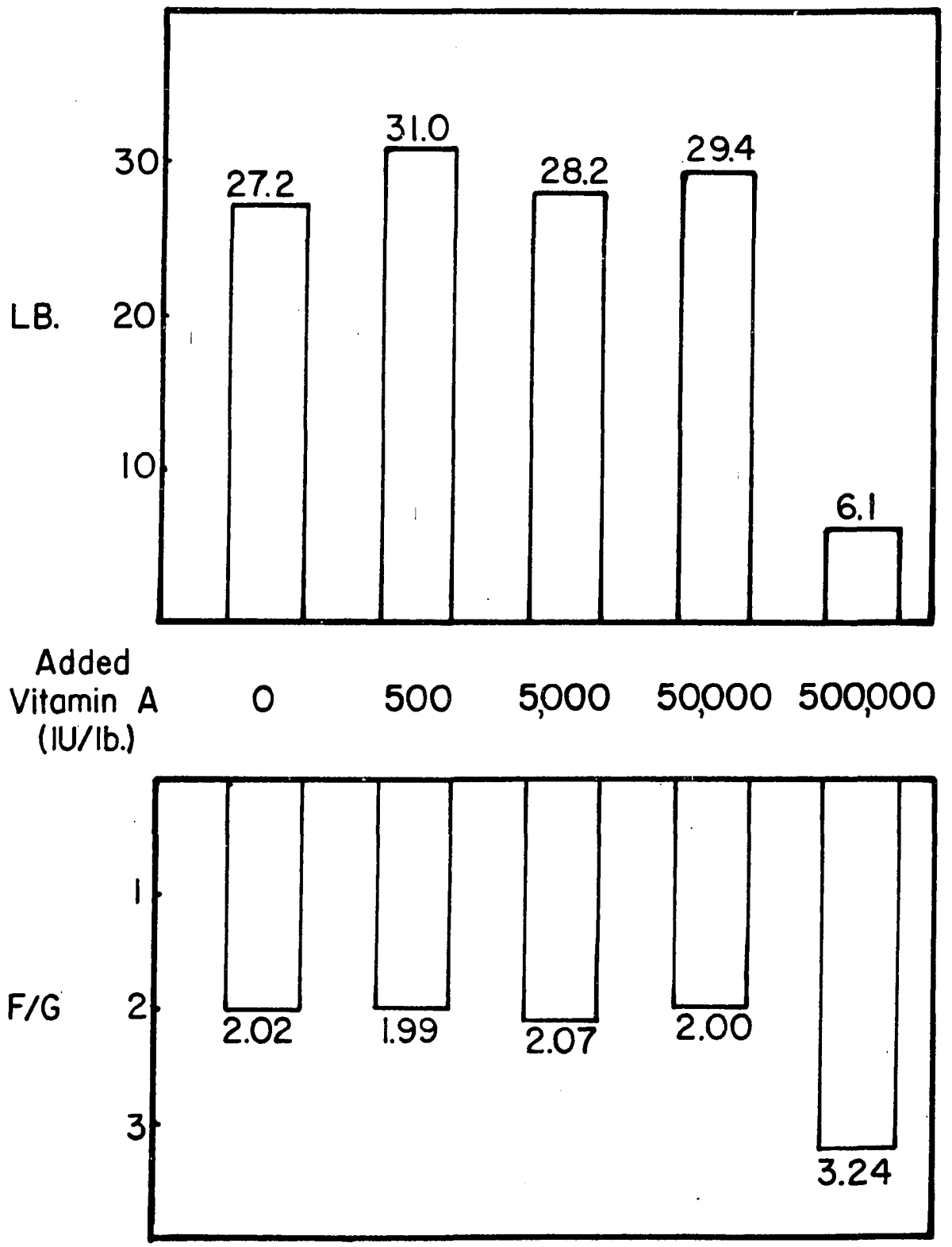


severely affected pigs which survived after four weeks on the experimental diet. This pig was unable to rise on his hind legs. Any effort to move appeared painful. Periodic tremors engulfed the pig. Figure 2 is an example of a pig less severely affected although on the same diet as the pig in Figure 1. More visible in this photograph are the petechial hemorrhages on the rear legs. The tendency to stand with back highly arched and rear legs planted in the anterior direction seemed to be a frequent characteristic of the condition. Pigs in Figure 3 are typical of those on all other treatments. They are vigorous, alert, and considerably larger than those with hypervitaminosis A.

The pigs shown in Figures 1 and 2 recovered after approximately three weeks on a standard diet, however five months later they had not yet reached an average weight of more than 150 pounds. The skeleton, especially the long bones of the legs, appeared to be permanently stunted, but at the same time thicker or larger in cross sectional area than those of normal pigs.

Summaries of weight gain and feed per pound gain are presented graphically in Figure 4 and in tabular form, together with average daily feed consumption, in Table 8 in the Appendix. The analysis of variance plan and a breakdown of the statistical analysis for these measurements are given in Table 9.

Figure 4. Experiment 1179 - Effect of vitamin A on weight gain (upper graph) and feed required per pound of gain (lower graph)


LB.
30
20
10
27.2
31.0
28.2
29.4
6.1
Added Vitamin A (IU/lb.)
0
500
5,000
50,000
500,000
F/G
1
2
3
2.02
1.99
2.07
2.00
3.24

The largest body weight gains were made by those pigs receiving diets with 500 IU of added vitamin A per pound of feed and these gained an average of 3.75 pounds per pig more than those receiving diets with no added vitamin A. The pigs on the highest level of vitamin A gained an average of only 6.1 pounds in four weeks. Statistical analysis revealed there was a significant linear, quadratic ($P = .01$) and higher polynomial response on weight gain as the logarithm of the dose (log dose) increased. Feed conversion ratio or feed required per pound of gain was significantly increased ($P = .05$) by the highest level of the vitamin used. Significant linear and quadratic effects were noted in average daily feed consumption ($P = .05$).

Metatarsal bones were cleaned of adhering tissue, weighed, dried at 100°C. for 36 hours and reweighed to calculate bone dry matter percent. Bone ether extract and ash were determined by standard methods. Serum calcium was determined by the method of Clark and Collip (1925) and serum inorganic phosphorus, as well as bone ash phosphorus, according to Fiske and Subbarow (1925). Bone ash calcium was determined gravimetrically by a method outlined by Diehl and Smith (1952). Serum and bone alkaline phosphatase analysis was carried out in the manner described by Liebholz (1963) as adapted from Lowry *et al.* (1954).

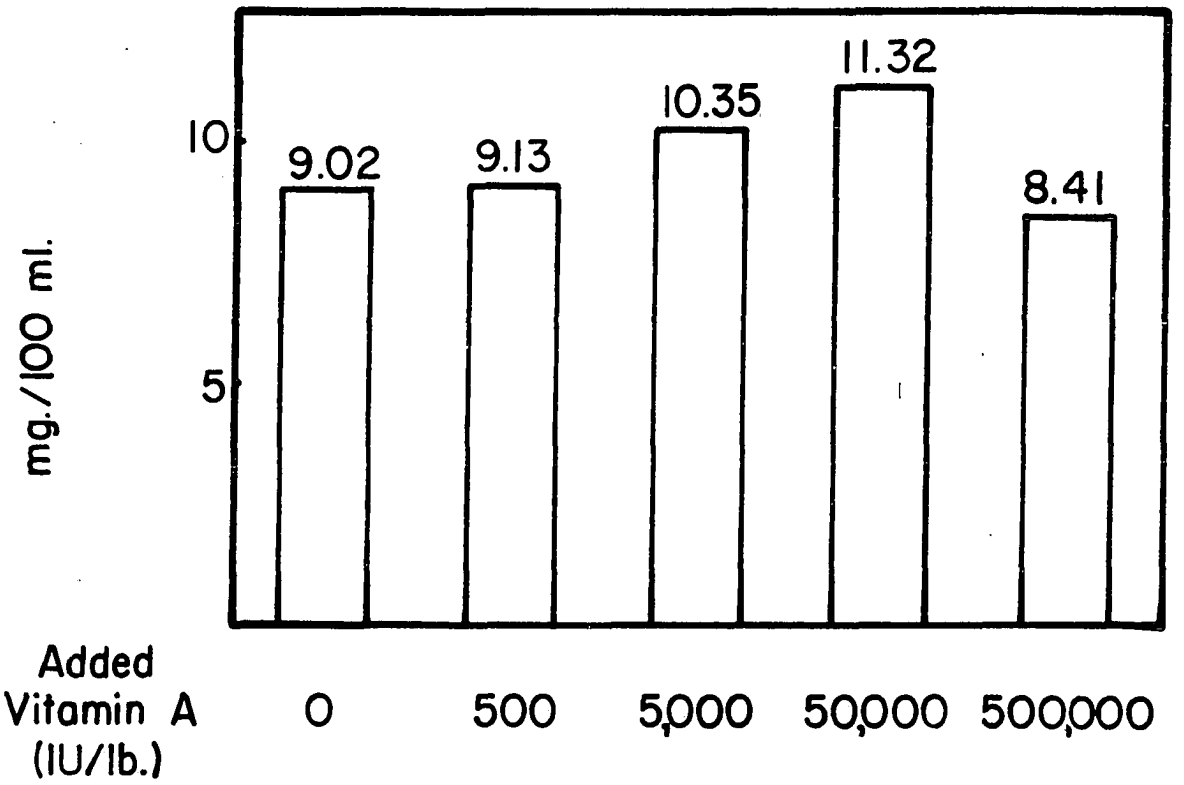
The results of serum inorganic phosphorus determinations

are illustrated in Figure 5 as are those for bone ash phosphorus. As the log dose increased, serum inorganic phosphorus tended to increase except at the highest level of vitamin A where it was depressed, resulting in a significant quadratic and cubic effect ($P = .05$). The addition of 500 IU of vitamin A per pound of diet depressed the phosphorus content of bone ash, but as the log dose of vitamin A increased above this, the percent phosphorus in bone ash increased. Overall, there was a significant linear ($P = .01$) and quadratic ($P = .05$) effect on bone ash phosphorus. Serum calcium and bone calcium concentrations are graphically illustrated in Figure 6. Serum calcium content varied between treatments considerably, but no statistically significant effects were discernable as was the case with bone ash percent calcium.

Serum alkaline phosphatase content was expressed as millimoles of nitrophenol liberated from the buffered-substrate, 2-amino-2-methyl propanol-nitrophenolphosphate (pH 9.8), per hour per 100 milliliters of serum. Bone was chopped to fine particles and leached in distilled water (5% bone, 95% water by weight) for 48 hours. One milliliter of the resulting liquid was diluted with ten milliliters of water and a portion of this solution analyzed for alkaline phosphatase in the same manner as was done for serum.

Figure 7 illustrates the effect of increasing dietary levels of vitamin A on serum and bone alkaline phosphatase.

Figure 5. Experiment 1179 - Effect of vitamin A on serum inorganic phosphorus (upper graph) and percent phosphorus in bone ash (lower graph)


mg./100 ml.
10
5
9.02
9.13
10.35
11.32
8.41
Added Vitamin A (IU/lb.)
0
500
5,000
50,000
500,000

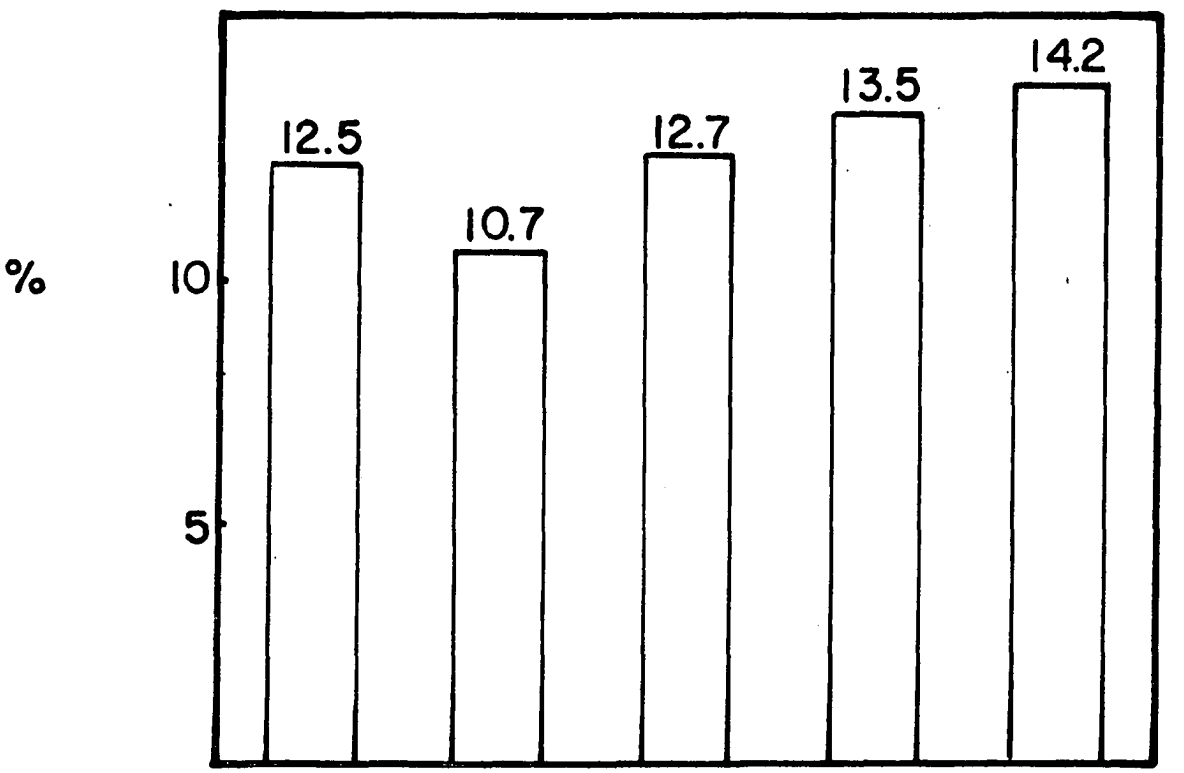
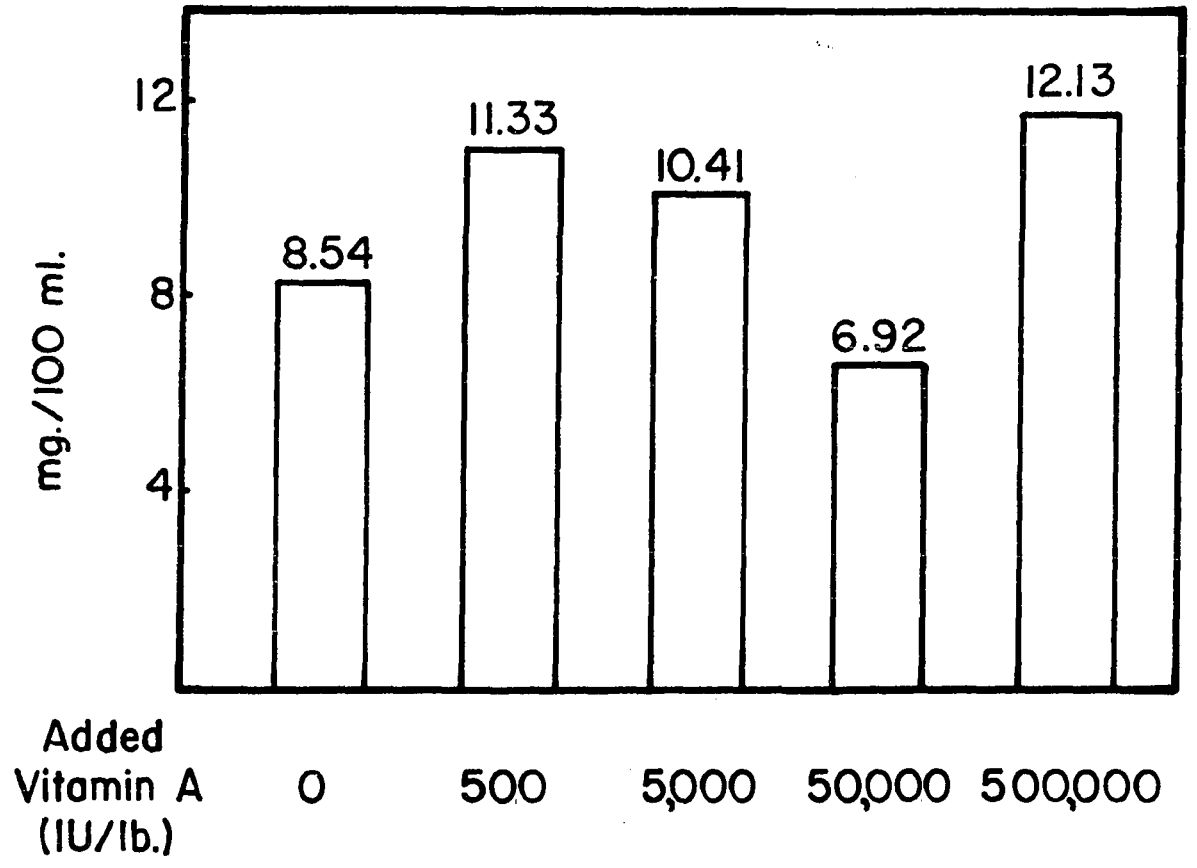

%
10
5
12.5
10.7
12.7
13.5
14.2

Figure 6. Experiment 1179 - Effect of vitamin A on serum calcium (upper graph) and percent calcium in bone ash (lower graph)


12
8
4
mg./100 ml.
8.54
11.33
10.41
6.92
12.13
Added Vitamin A (IU/lb.)
0
500
5,000
50,000
500,000

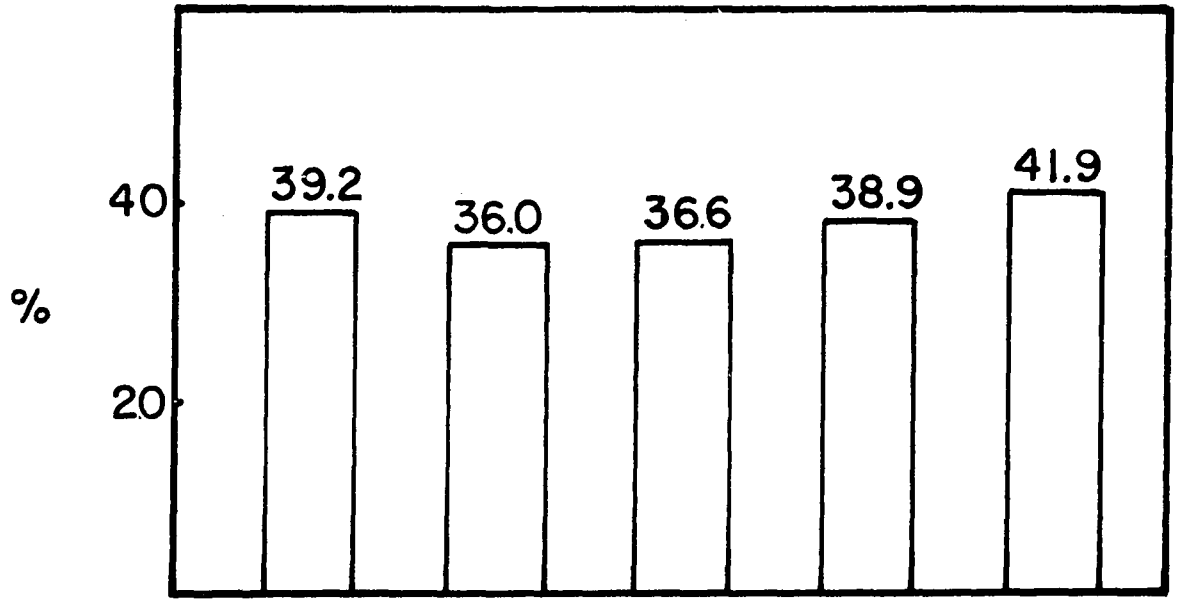
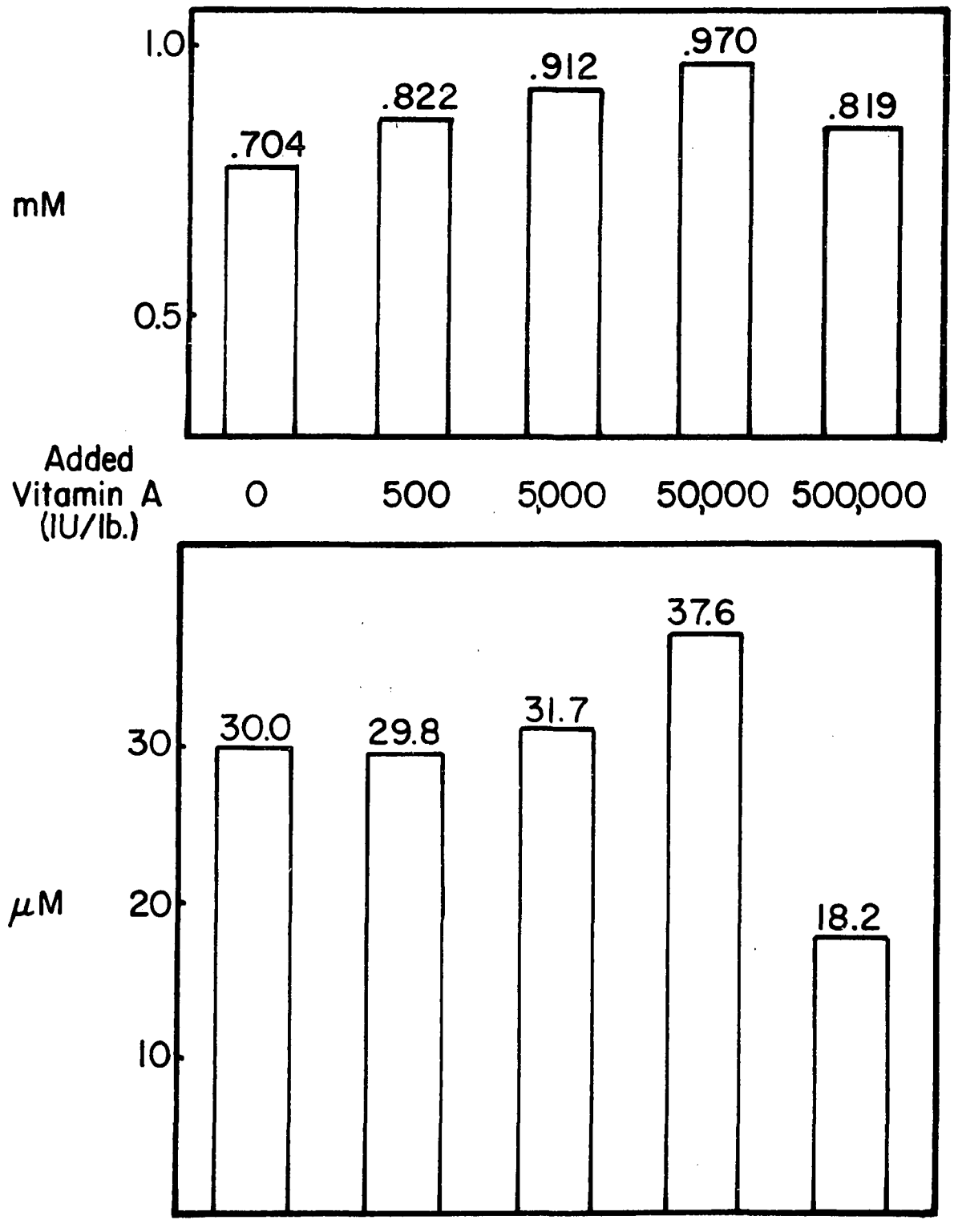

40
20
%
39.2
36.0
36.6
38.9
41.9

Figure 7. Experiment 1179 - Effect of vitamin A on serum alkaline phosphatase activity (upper graph) and on bone alkaline phosphatase activity (lower graph)

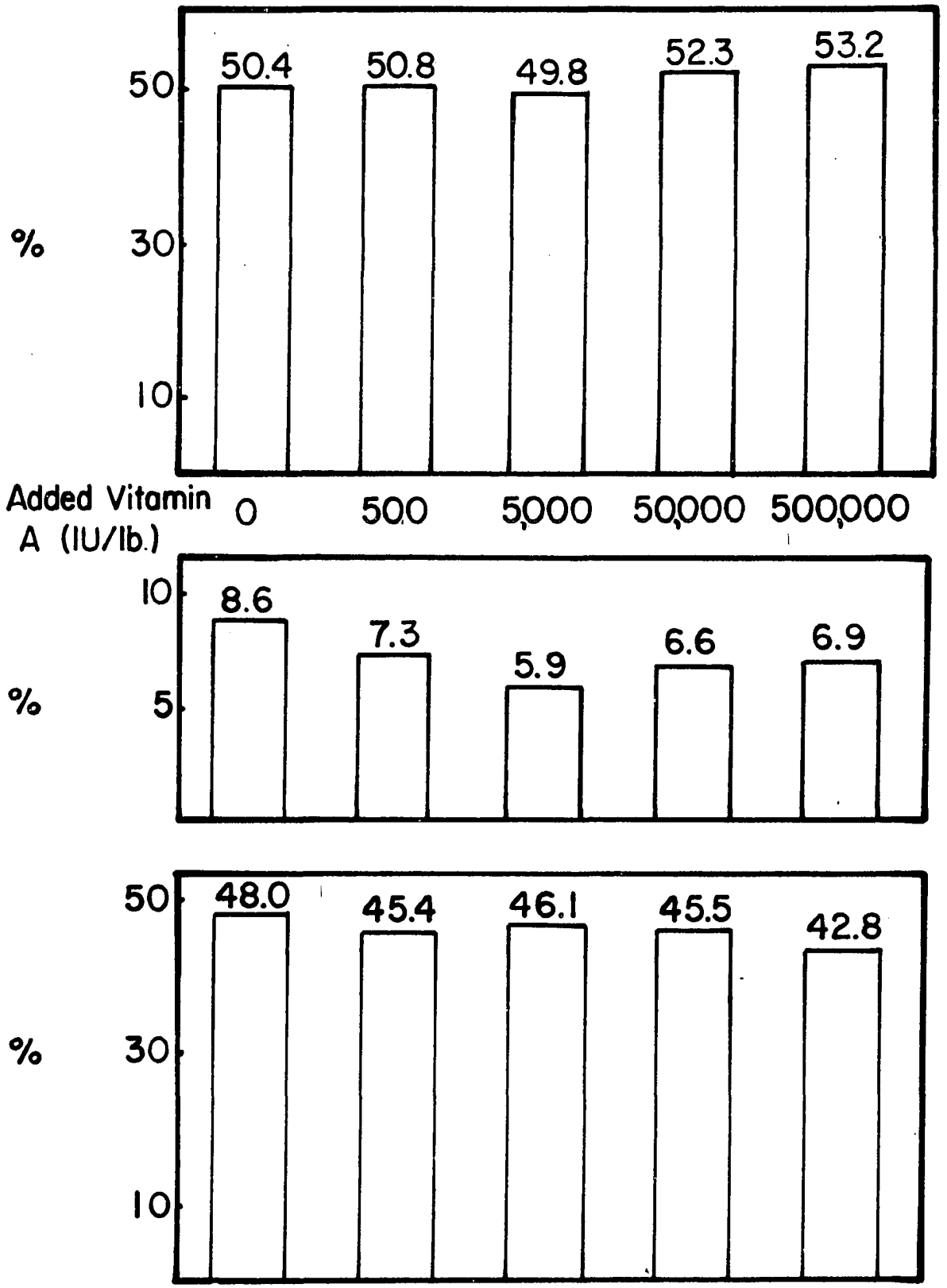

1.0
0.5
mM
.704
.822
.912
.970
.819
Added Vitamin A (IU/lb.)
0
500
5,000
50,000
500,000
30
20
10
μM
30.0
29.8
31.7
37.6
18.2

In general, in both serum and bone the alkaline phosphatase activity tended to increase as the log dose increased, except at the highest dietary level of the vitamin where the enzyme activity declined, especially in bone. No significant statistical effect was established on alkaline phosphatase activity of either bone or of blood serum. The results of bone dry matter, bone ether extract, and the ash of dry, fat-free bone are illustrated in Figure 8. As the log dose increased, there was a significant linear increase ($P = .05$) in bone dry matter percentage, and a decrease in ether extract (linear quadratic, and cubic). Average percent ash in bone decreased markedly with increased vitamin A, but this response was not statistically significant due to the range of values determined between pigs on the same treatment.

Values from individual pigs for serum calcium, inorganic phosphorus, and alkaline phosphatase are found in Table 10 in the Appendix. Those for bone ash calcium and phosphorus, and bone alkaline phosphatase are given in Table 11, while the data for bone dry matter, ether extract, and ash are located in Table 14. Organization of the analysis of variance and the breakdown of the statistical analysis for these determinations are given in Tables 12, 13, and 15.

An attempt was made to find a relationship between several blood and bone components as they may be affected by hypervitaminosis A by calculation of correlation coefficients.

Figure 8. Experiment 1179 - Effect of vitamin A on percent dry matter in bone (upper graph), percent ether extract in bone (middle graph), and percent ash in bone (lower graph)


50.4
50.8
49.8
52.3
53.2
50
30
10
%
Added Vitamin A (IU/lb.)
0
500
5,000
50,000
500,000
8.6
7.3
5.9
6.6
6.9
10
5
%
48.0
45.4
46.1
45.5
42.8
50
30
10
%

These are found in Table 1. The only statistically significant correlation found was that between bone ash phosphorus and calcium which one would expect to be closely related. This suggests that hypervitaminosis A may affect the structure

Table 1. Experiment 1179 - Correlation coefficients calculated with data from all pigs on experiment

Variables	Coefficient
Bone ash phosphorus vs. bone ash calcium	0.8151[a]
Serum calcium vs. bone ash calcium	0.2886
Serum inorganic phosphorus vs. bone ash phosphorus	0.1426
Serum inorganic phosphorus vs. serum calcium	-0.3190
Serum alkaline phosphatase vs. bone alkaline phosphatase	0.2509
Bone alkaline phosphatase vs. bone ash phosphorus	-0.2054
Bone alkaline phosphatase vs. bone ash calcium	-0.2716
Serum alkaline phosphatase vs. bone ash phosphorus	-0.1957
Serum alkaline phosphatase vs. serum inorganic phosphorus	0.2324

[a]Significant at P = .01.

and function of bone, since the calcium and phosphorus contents of bones are influenced in the same direction. The correlation coefficient between serum and bone calcium was larger than that between serum inorganic phosphorus and bone ash phosphorus which may be due to 1) different rates of clearance of the minerals from the blood, 2) blood phosphorus content being influenced by body organs and tissues other than bone to a greater extent than is serum calcium, or 3) other

factors.

Serum inorganic phosphorus and serum calcium seem to be inversely related ($r = -0.3190$), supporting the concept of calcium-phosphorus solubility product maintenance in the blood. Alkaline phosphatase enzyme activity in the blood was not significantly correlated to that of bone in this experiment, suggesting that perhaps if serum alkaline phosphatase reflects the action of hypervitaminosis A on bone, the metatarsal bones are perhaps not a good indicator of this.

Concentrations of alkaline phosphatase activity in bone and in serum are negatively correlated to the phosphorus content of the bone ash in the same order of magnitude, but serum alkaline phosphatase was positively correlated with serum inorganic phosphorus.

Experiment 6327 - The effect of vitamin D on a high level of vitamin A

Objectives This experiment was conducted to determine what effects a high dietary level of vitamin A would produce in pigs fed zero or small amounts of vitamin D.

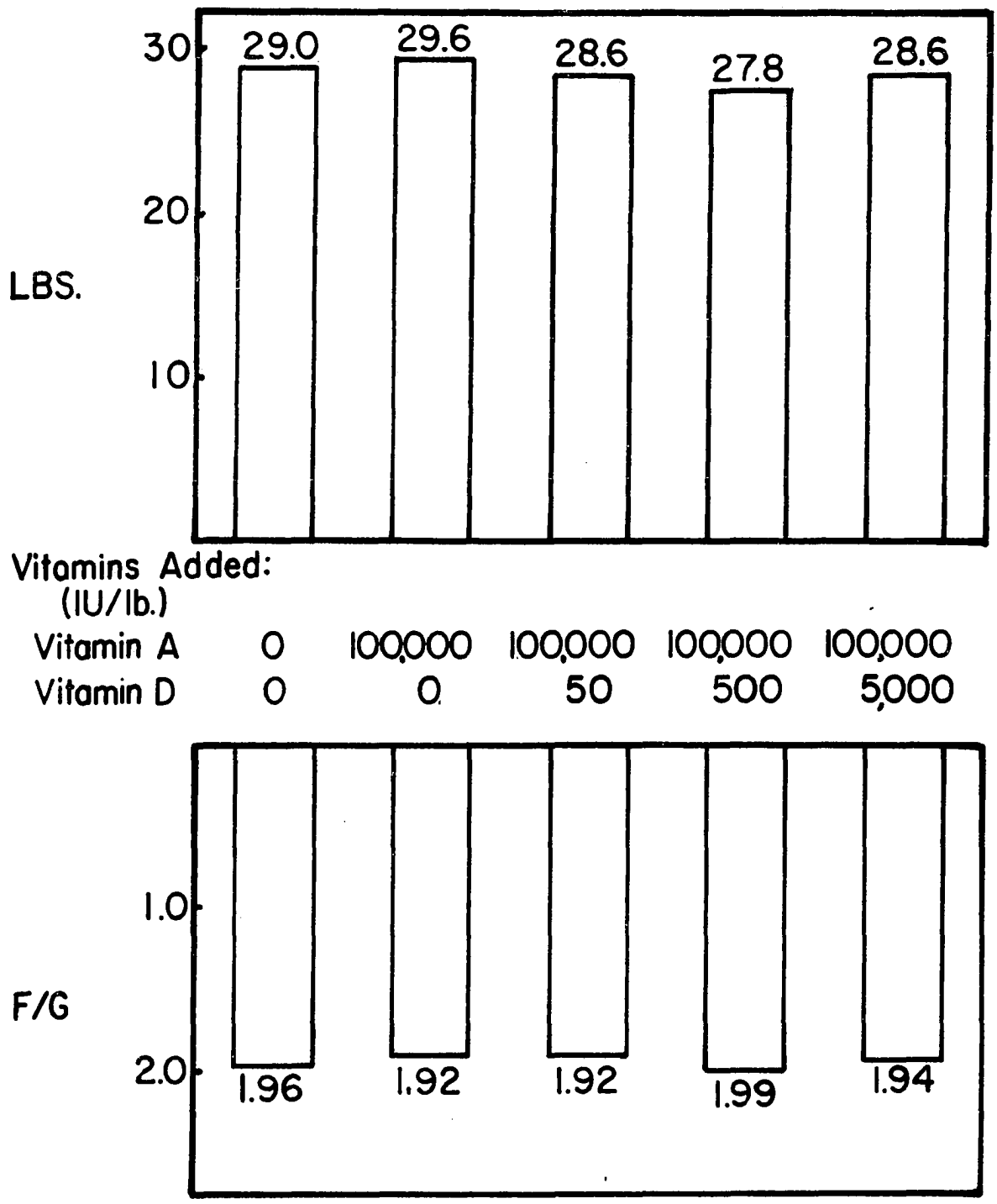
Experimental Forty pigs averaging 9.7 pounds body weight and 16.6 days of age were allotted, four pigs per pen, and fed the experimental diets for a 42-day period. The treatments were 0 or 100,000 IU of added vitamin A per pound with no vitamin D, and 100,000 IU of added vitamin A per pound with 0, 50, 500, or 5,000 IU of vitamin D per pound.

This experiment was conducted during June and July of 1963 in unit A, previously described. Feed and water were provided ad libitum. No heat lamps were used at any time during the six-week experiment. Weight gain and feed consumption were determined and recorded at weekly intervals. Blood samples were drawn and the serum enzyme analyses made after the pigs were on the experimental diets 40 days. Pigs were weighed off experiment on day 42, and metatarsal bones subsequently removed. The composition of the basal ration is found in Table 4 in the Appendix.

Results and discussion Summaries of weight gain and feed per pound gain are presented in Figure 9 and Table 16. The analysis of variance plan and the breakdown of the statistical analysis are shown in Table 17. There were no significant effects on weight gain or feed required per pound of gain due to ration treatment.

Summaries of serum calcium and inorganic phosphorus are found in Table 18, and data for serum alkaline and acid phosphatase in Table 19. Serum acid phosphatase determinations were made with an acetate buffer-substrate solution of pH 4.8 similar to that used by Sprague et al. (1963). Serum calcium in this and all subsequent experiments was determined by the method of Ferro and Hamm (1957). Analysis of variance breakdown and calculated mean squares for the preceding determinations are given in Table 20. Average serum calcium and phos-

Figure 9. Experiment 6327 - Effect of vitamins A and D on pounds of gain (upper graph) and feed required per pound of gain (lower graph)

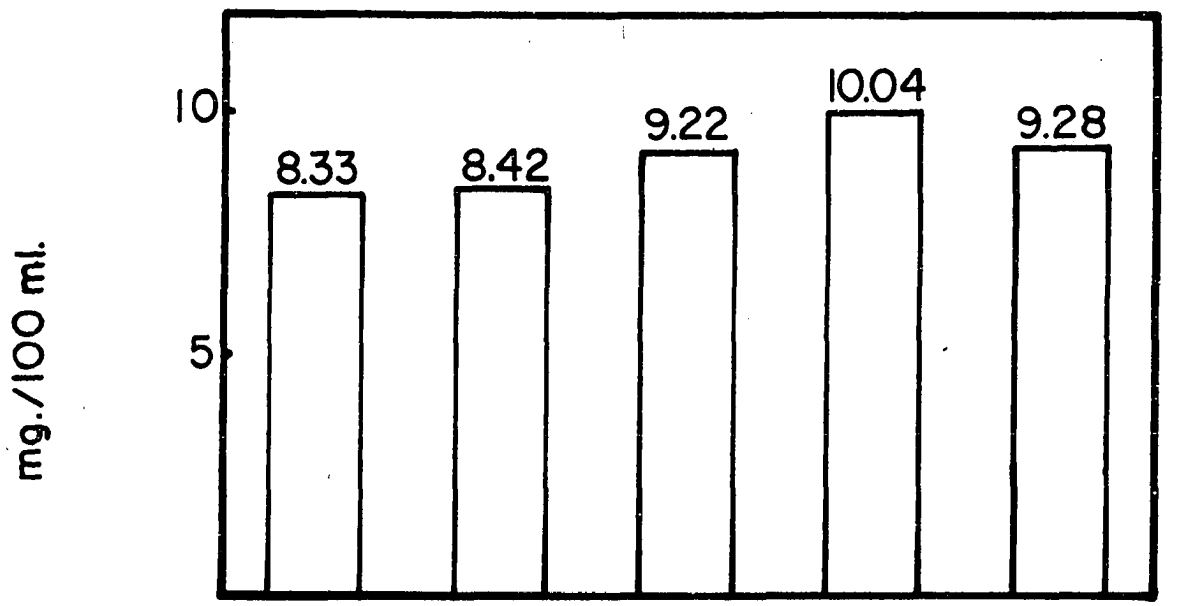

30
20
10
LBS.
29.0
29.6
28.6
27.8
28.6
Vitamins Added:
(IU/lb.)
Vitamin A 0 100,000 100,000 100,000 100,000
Vitamin D 0 0 50 500 5,000
1.0
2.0
F/G
1.96
1.92
1.92
1.99
1.94

phorus values are shown graphically in Figure 10. The addition of 100,000 IU of vitamin A per pound to the diet caused a significant (P = .01) increase in serum calcium and also in serum inorganic phosphorus. There was a significant linear and quadratic response in serum calcium levels to increasing dietary levels of vitamin D (P = .01).

Figure 11 illustrates the average values determined for serum alkaline phosphatase and serum acid phosphatase activity by treatment. Vitamin A (100,000 IU/lb.) added to the diet significantly (P = .01) depressed serum alkaline phosphatase activity. The added vitamin A also depressed serum acid phosphatase activity, but this effect was not statistically significant. The activity of either serum enzyme was not affected by dietary level of vitamin D.

Summaries of bone alkaline phosphatase and bone acid phosphatase are given in Table 21. Bone ether extract and bone ash (percent ash of dry, fat-free bone) percentages are found in Table 22. Analysis of variance plans and breakdown of the statistical analysis for these determinations are found in Table 23. Average bone alkaline and acid phosphatase activity is graphically illustrated in Figure 12. The addition of 100,000 IU of vitamin A per pound of diet of the pigs resulted in significantly less bone alkaline phosphatase (P = .05). The added vitamin A increased average bone acid phosphatase except when 50 IU of vitamin D per pound of diet

Figure 10. Experiment 6327 - Effect of vitamins A and D on serum calcium (upper graph) and serum inorganic phosphorus (lower graph)



Vitamins Added:
(IU/lb.)

Vitamin A	0	100,000	100,000	100,000	100,000
Vitamin D	0	0	50	500	5,000

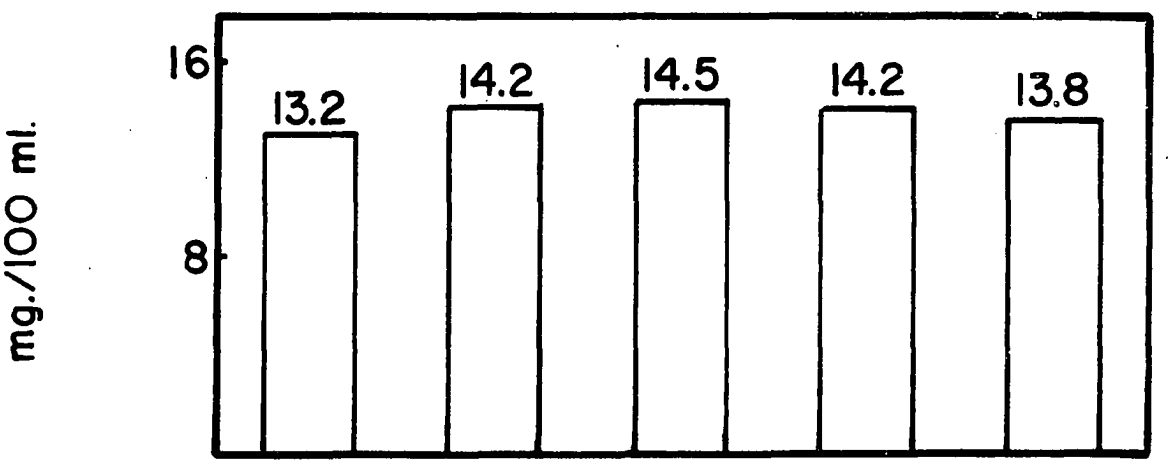
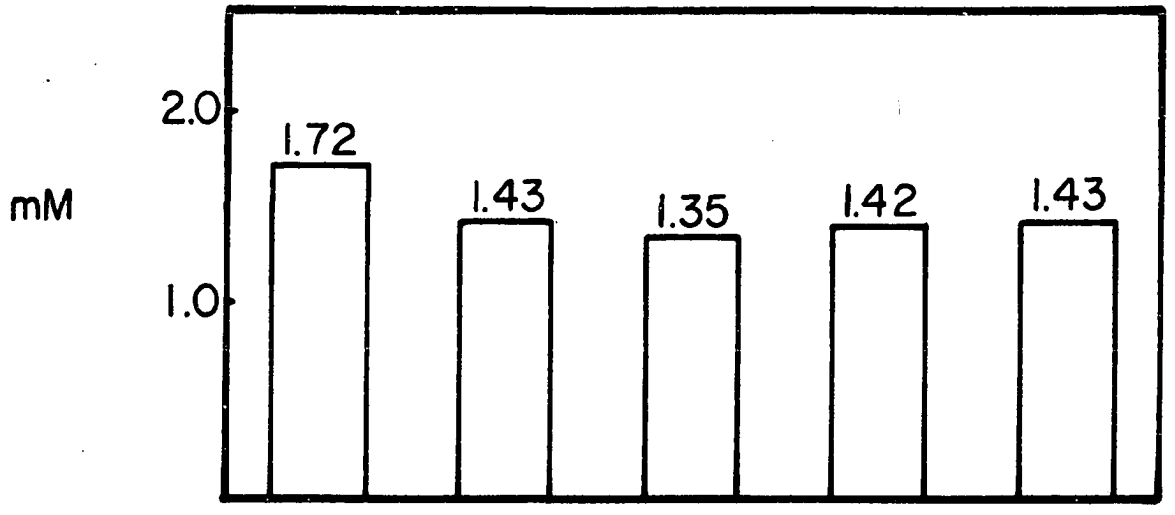


Figure 11. Experiment 6327 - Effect of vitamins A and D on serum alkaline phosphatase activity (upper graph) and serum acid phosphatase activity (lower graph)



Vitamins Added:
(IU/lb.)

Vitamin A	0	100,000	100,000	100,000	100,000
Vitamin D	0	0	50	500	5,000

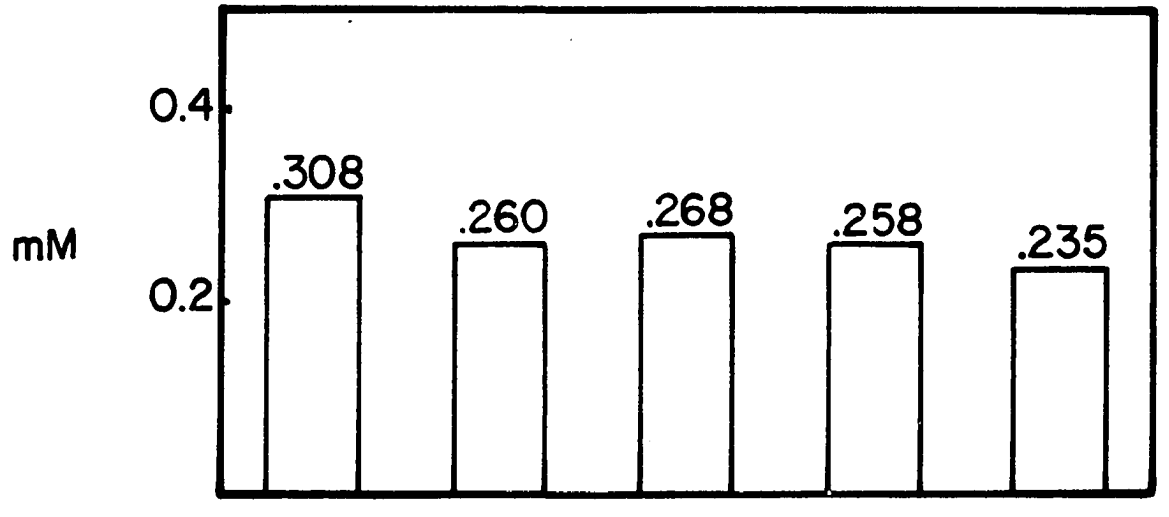
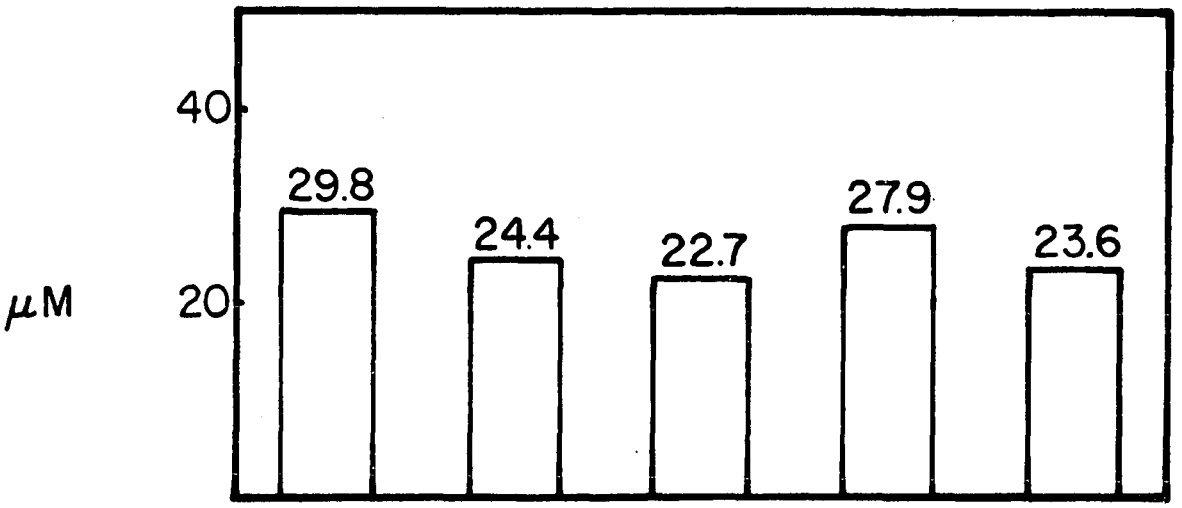
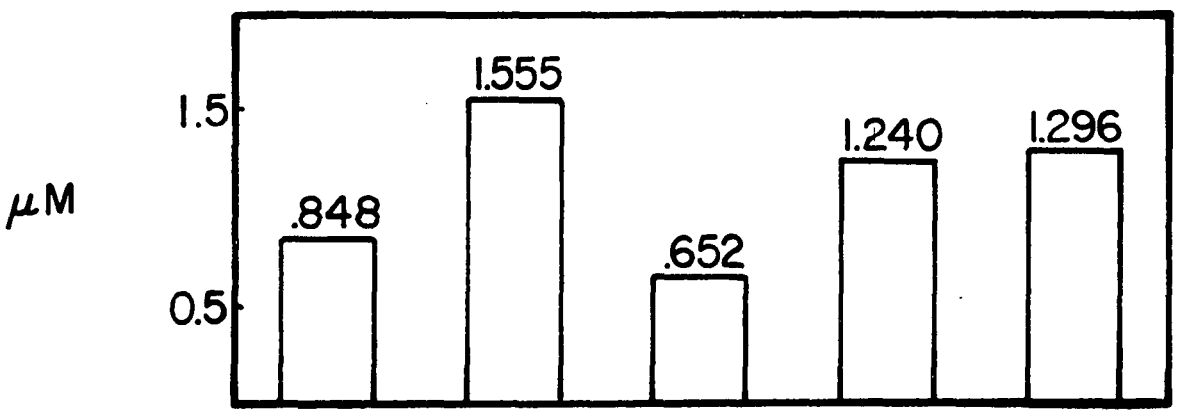


Figure 12. Experiment 6327 - Effect of vitamins A and D on bone alkaline phosphatase activity (upper graph) and on bone acid phosphatase activity (lower graph)



Vitamins Added:
(IU/lb.)

Vitamin A	0	100,000	100,000	100,000	100,000
Vitamin D	0	0	50	500	5000



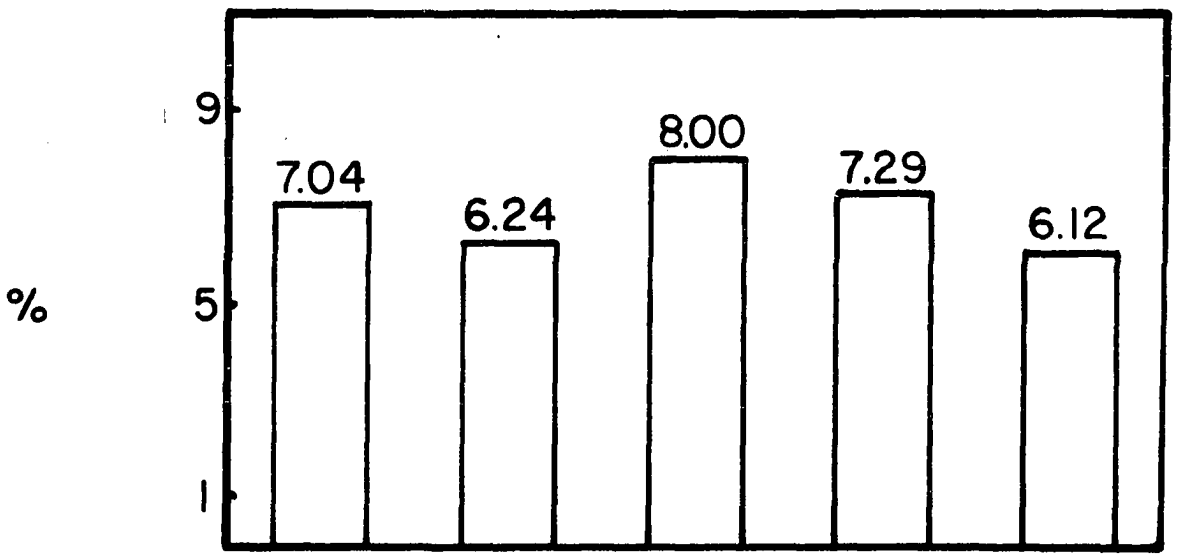
also were fed; however, no statistically significant effects were detected in this analysis which could be ascribed to dietary treatment.

Average bone ether extract and bone ash results are graphically given in Figure 13. No statistically significant dietary effects were detected with either of these determinations.

On ashing, the bone epiphysis and diaphysis sections separated, and these parts were analyzed separately. Bone ash was dissolved in 2N HCL and the resulting solutions analyzed for calcium volumetrically. To a sample of the dissolved and diluted bone ash was added a color indicator consisting of 0.5 gram Eriochrome black T dissolved in 100 ml of diethanolamine. An amount of magnesium solution of precisely known concentration was added to the unknown solution and then a solution of disodium EDTA (ethylenediamine tetraacetic acid) of known molarity was titrated in until the indicator changed color. From known amounts of the standard solutions of magnesium and also the EDTA solution the calcium content of the sample portion used could be calculated. Phosphorus in the bone ash solutions was again determined by the method of Fiske and Subbarow (1925).

Epiphysis and diaphysis calcium concents are given in Table 24 and the values for phosphorus in Table 25. These are illustrated graphically in Figures 14 and 15, respectively.

Figure 13. Experiment 6327 - Effect of vitamins A and D on bone ether extract (upper graph) and bone ash (lower graph)



Vitamins Added:
(IU/lb.)

Vitamin A	0	100,000	100,000	100,000	100,000
Vitamin D	0	0	50	500	5,000

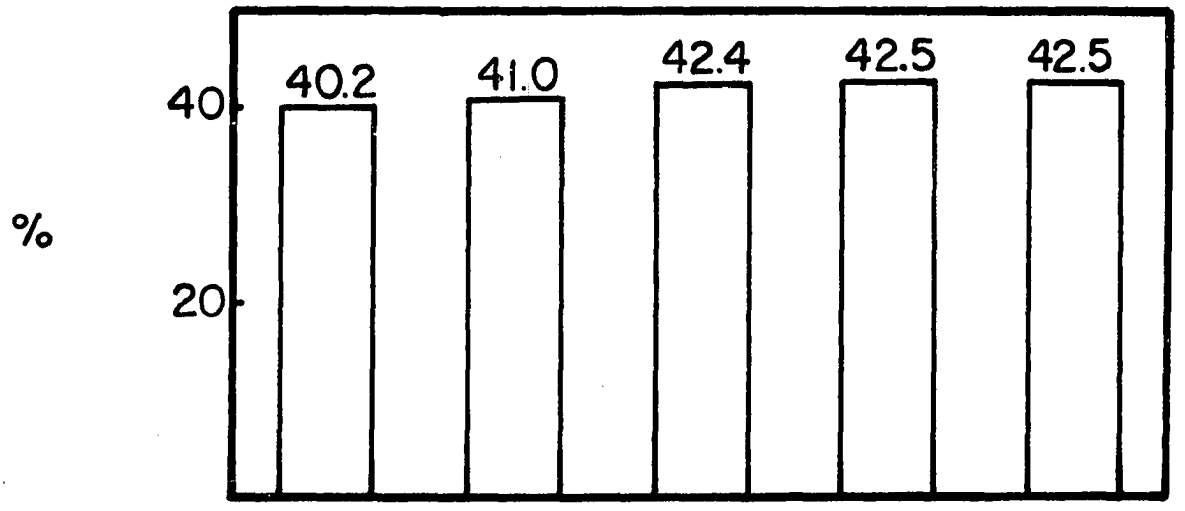
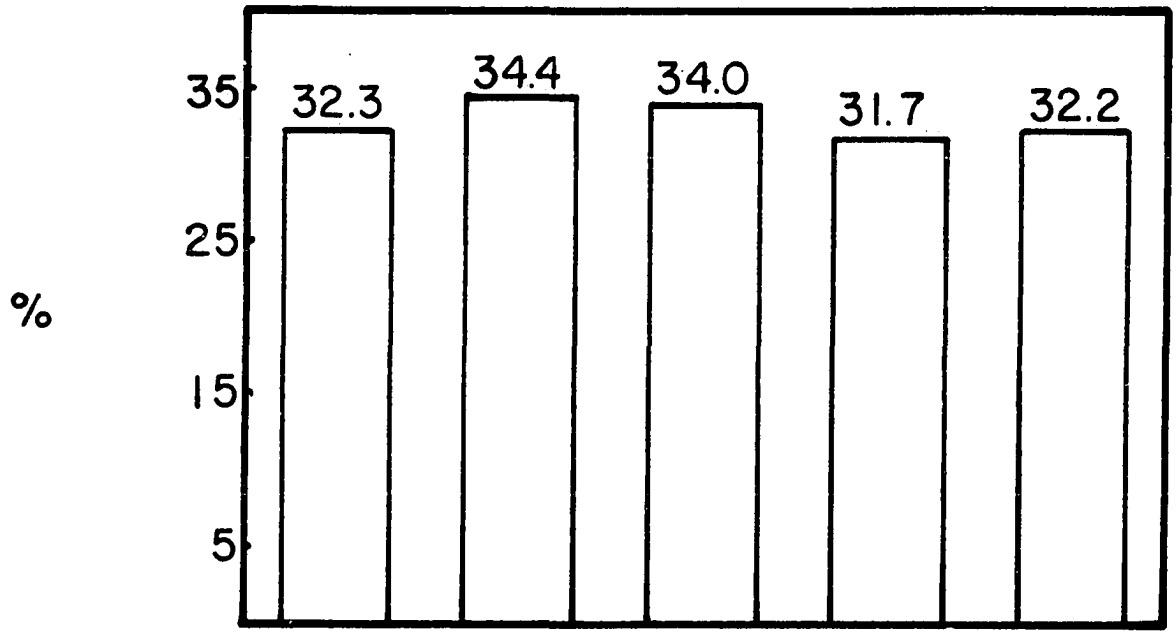


Figure 14. Experiment 6327 - Effect of vitamins A and D on percent calcium in diaphysis bone ash (upper graph) and on epiphysis bone ash (lower graph)



Vitamins Added:
(IU/lb.)

Vitamin A	0	100,000	100,000	100,000	100,000
Vitamin D	0	0	50	500	5,000

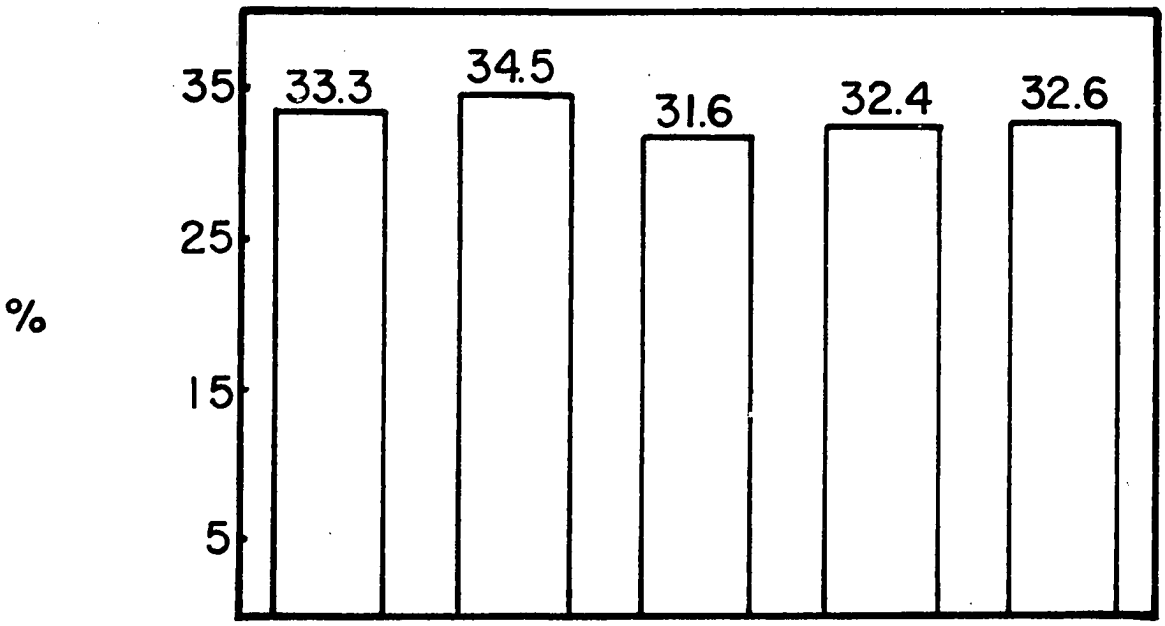
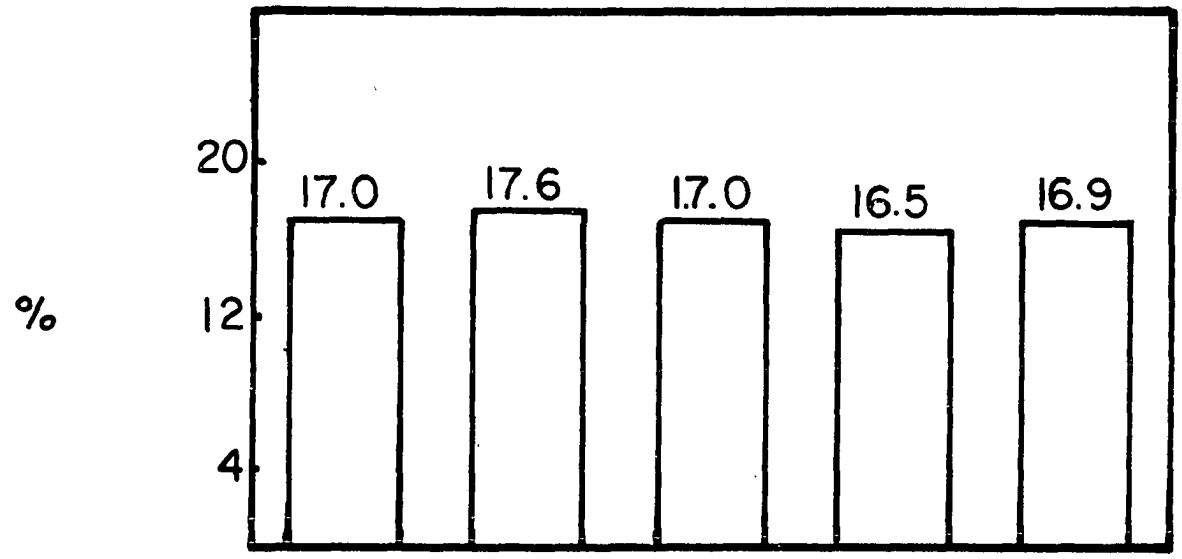
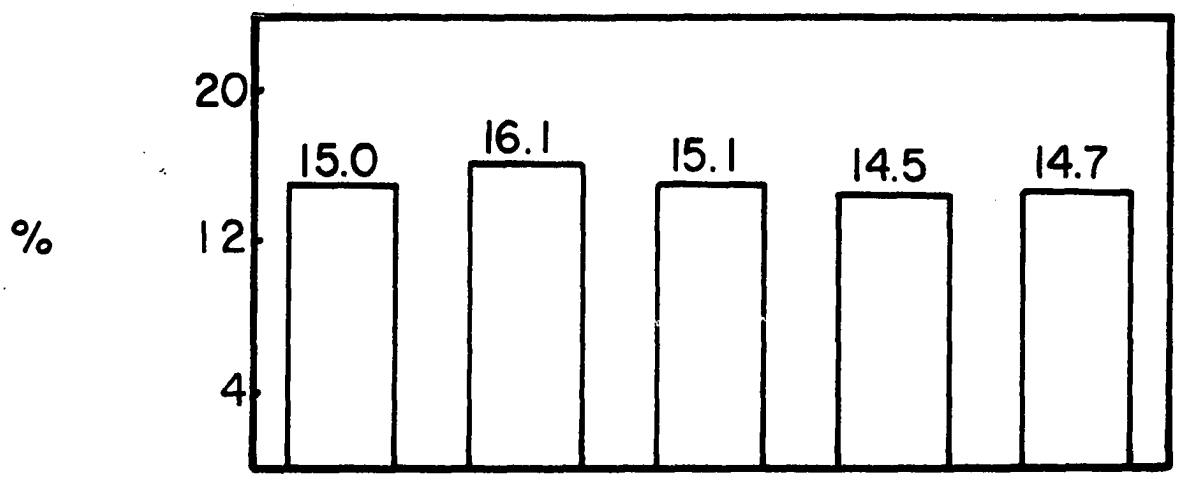


Figure 15. Experiment 6327 - Effect of vitamins A and D on percent phosphorus in diaphysis bone ash (upper graph) and in epiphysis bone ash (lower graph)



Vitamins Added:
(IU/lb.)

Vitamin A	0	100,000	100,000	100,000	100,000
Vitamin D	0	0	50	500	5,000



Analysis of variance breakdown and calculated mean squares are given in Table 26. No significant treatment effects were noted in any of these determinations. Diets containing 100,000 IU of added vitamin A per pound with no vitamin D resulted in the highest percent calcium and phosphorus in the bone ash although, as shown in Figure 13, bones from pigs on this treatment contained a lower percent ash than those from pigs receiving dietary vitamin D. Epiphyseal ash contained a lower percentage phosphorus than diaphyseal ash although epiphyseal and diaphyseal calcium contents (percent) were nearly the same.

At no time during the six-week experimental period were any symptoms of vitamin D deficiency evident in those pigs receiving diets containing no added vitamin D or any other diet. Symptoms suggestive of vitamin A toxicity did appear, however, in some pigs as evidenced by their external appearance. Two pigs died after approximately four weeks on experiment. One of these had definite symptoms of vitamin A toxicity. Postmortem examination revealed anemia, widened costochondral junctions, hemoperitoneum and hydropericardium, friable areas in the liver and fenestration of Glisson's capsule. No etiological agent was isolated from the pigs and death was ascribed to hypervitaminosis A. No pigs on the highest level of vitamin D (5,000 IU/lb.) died.

A number of correlation coefficients were calculated in

an attempt to detect relationships between variables influenced by the dietary treatments. These coefficients are given in Table 2. Only five of the calculated coefficients are statistically significant, however the sign and magnitude of some of those found not significant in this experiment are of interest.

There was a significant positive correlation between serum alkaline phosphatase and bone alkaline phosphatase concentrations, but a negative, though not significant, correlation between serum and bone acid phosphatase contents. Serum acid phosphatase and serum alkaline phosphatase were, however, significantly positively correlated suggesting that the acid phosphatase of the serum is not, to any appreciable extent, derived from bone or that the enzyme in the metatarsal bones is not a satisfactory indicator of the skeletal content of the enzymes under these experimental conditions.

Serum inorganic phosphorus was more highly correlated to serum acid phosphatase than to serum alkaline phosphatase, but the inorganic phosphorus content in the serum was negatively correlated to both these phosphatase enzymes as found in bone.

That there was a positive correlation between the percent calcium and percent phosphorus in diaphysis bone ash but a negative correlation of these in epiphysis bone ash is possibly due to the growth activity taking place in the epiphysis

Table 2. Experiment 6327 - Summary of correlation coefficients

Variables	Coefficient
Serum acid phosphatase vs. serum alkaline phosphatase	0.3918[a]
Serum inorganic P vs. serum acid phosphatase	0.3691[a]
Serum alkaline phosphatase vs. bone alkaline phosphatase	0.3390[a]
Serum inorganic phosphorus vs. epiphysis ash P	0.2766
Bone alkaline phosphatase vs. epiphysis ash P	0.2685
Serum acid phosphatase vs. epiphysis ash P	0.2020
Serum alkaline phosphatase vs. epiphysis ash P	0.1547
Serum calcium vs. serum inorganic phosphorus	0.1499
Serum inorganic phosphorus vs. diaphysis ash P	0.1415
Diaphysis ash phosphorus vs. diaphysis ash calcium	0.1200
Serum inorganic P vs. serum alkaline phosphatase	0.1163
Bone acid phosphatase vs. bone alkaline phosphatase	0.0452
Serum alkaline phosphatase vs. diaphysis ash P	0.0429
Serum acid phosphatase vs. diaphysis ash P	-0.0071
Serum Ca vs. diaphysis ash Ca	-0.0509
Bone ether extract vs. bone acid phosphatase	-0.0561
Bone ether extract vs. bone alkaline phosphatase	-0.0603
Serum acid phosphatase vs. bone alkaline phosphatase	-0.0622
Serum Ca vs. bone alkaline phosphatase	-0.0763
Bone acid phosphatase vs. epiphysis ash P	-0.1045
Epiphysis ash P vs. epiphysis ash Ca	-0.1513
Serum Ca vs. epiphysis ash Ca	-0.1705
Serum acid phosphatase vs. bone acid phosphatase	-0.1918
Serum Ca vs. serum alkaline phosphatase	-0.1926
Serum inorganic P vs. bone alkaline phosphatase	-0.2164
Serum inorganic P vs. bone acid phosphatase	-0.2382
Serum alkaline phosphatase vs. bone acid phosphatase	-0.2909
Bone alkaline phosphatase vs. diaphysis ash P	-0.3276[a]
Bone acid phosphatase vs. diaphysis ash P	-0.3674[a]

[a]Significant at P = .05 or less.

or, since the average bone ash percentage increased with increased levels of vitamin D, these coefficients may be indicative of the differential rate of maturation of these bone parts.

Serum inorganic phosphorus was more highly correlated with epiphyseal ash phosphorus than diaphyseal ash phosphorus. There was essentially no correlation between phosphatase enzymes (acid and alkaline) in the blood serum and the diaphyseal ash phosphorus, but there was a certain positive correlation between these serum enzymes and the epiphyseal ash phosphorus content.

Bone alkaline phosphatase was correlated positively with epiphyseal ash phosphorus and significantly negatively correlated with diaphysis ash phosphorus. Bone acid phosphatase was significantly negatively correlated with diaphysis ash phosphorus also, but at the same time correlated negatively, although to a lesser extent, with epiphyseal ash phosphorus. It should be emphasized that most of the correlation coefficients discussed were not statistically significant in this experiment.

Experiment 6311 - Effect of vitamin D on a high level of vitamin A

Objectives The purpose of this experiment was to determine if certain dietary levels of vitamin D would alter the effect of a high dietary level of vitamin A in regard to

weight gain, feed required per pound of gain, serum calcium, and inorganic phosphorus, and serum alkaline and acid phosphatase.

Experimental Forty-eight pigs averaging 9.8 pounds and 16.4 days of age were allotted, four pigs to the pen. One replication was confined to pens in unit A, previously described; the second replication was confined to pens in unit C. This unit provided concrete-floored pens which were covered with wood shavings. Self-feeders and automatic waterers were also provided, but the water supplied in this facility was de-ionized by passing it through an ion-exchange apparatus as part of other experiments being conducted in this building.

This experiment was conducted during March, April, and May of 1963 and each replication was of six weeks duration. Heat lamps were used only during the first two weeks of the experiment. This experiment was of randomized block design with a 2 x 3 factorial arrangement of treatments which were 0 or 100,000 IU of added vitamin A with either 500, 5,000, or 50,000 IU of vitamin D added per pound of diet. Feed and water were provided ad libitum.

Animal weights and feed consumption were determined weekly. Serum calcium, inorganic phosphorus, alkaline phosphatase activity, and acid phosphatase activity determinations were made at the end of the six-week experimental period. The composition of the basal ration is given in Table 4 of the

Appendix.

Results and discussion Summary data of total weight gain and feed per pound gain are given in Table 27 in the Appendix. Analysis of variance breakdown and calculated mean squares are found in Table 28. The addition of 100,000 IU of vitamin A to the diet significantly (P = .01) depressed weight gains. There were no significant treatment effects on weight gain due to vitamin D. Feed required per pound of gain for the pigs on all rations was essentially the same.

The summary of serum calcium and serum inorganic phosphorus determinations is given in Table 29. Increasing the level of vitamin D in the basal diet progressively increased average blood serum calcium content, but when the diet contained 100,000 IU of vitamin A per pound, this effect was nullified. There was a highly significant (P = .01) difference in serum calcium between replications. Pigs which received de-ionized water (Replication 1) had markedly less blood serum calcium than those receiving tap water (Replication 2). Serum inorganic phosphorus was not significantly affected by either added vitamin D or vitamin A, however, again there was a significant difference between replications as those pigs which received the de-ionized water to drink had significantly lower levels of serum inorganic phosphorus than those drinking tap water.

Serum alkaline phosphatase and acid phosphatase data are

given in Table 30 in the Appendix, and the analysis of variance plan and breakdown of the statistical analysis for these determinations as well as for serum calcium and inorganic phosphorus are given in Table 31.

Serum alkaline phosphatase concentrations were significantly higher in those pigs in Replication 2 than those in Replication 1, possibly due indirectly to the mineral content of the water consumed. There was a significant ($P = .01$) interaction of vitamins A and D on alkaline phosphatase activity in the serum. This seems to be due to the anomalous concentrations of enzyme activity found in the serum of the pigs fed 5,000 IU of vitamin D per pound of feed. There were no significant treatment effects on serum acid phosphatase concentrations. No definite symptoms of hypervitaminosis A were observed; neither were there indications that 50,000 IU of vitamin D per pound of diet were detrimental to the pigs.

Experiment 6318 - A preliminary trial with a high level of vitamin A and vitamin C and/or thyroprotein

Objectives This experiment was conducted to determine if vitamin C and/or thyroprotein (iodinated casein), when fed with a diet containing 100,000 IU of added vitamin A per pound, had any influence on weight gain, feed required per pound of gain, and the blood serum components calcium, inorganic phosphorus, alkaline phosphatase, and acid phosphatase.

Experimental This experiment is a combination of similar trials made at different times. Group 1 (Replication 1) was placed on experiment for six weeks in April and May, 1963; Group 2 (Replications 2 and 3) was placed on experiment beginning in November, 1963).

Forty-eight pigs averaging 9.5 pounds and 19.0 days of age were allotted, four pigs per pen, and fed the experimental diets for a 42-day period. All diets contained 100,000 IU of added vitamin A per pound together with 0 or 100 mg of vitamin C per pound and 0 or 100 mg of thyroprotein per pound. The experiment was conducted in unit A, previously described. Feed and water were provided ad libitum. The composition of the basal ration is given in Table 4 in the Appendix.

Blood samples were drawn for analysis at the end of the 42-day experimental period.

Results and discussion Summaries of weight gain and feed required per pound of gain appear in Table 32 in the Appendix. The analysis of variance plan and breakdown of the statistical analysis are given in Table 33. There were no significant differences in weight gains due to ration treatment although average total gain was less in pigs fed diets containing either thyroprotein or vitamin C. The addition of 100 mg of thyroprotein per pound of diet significantly ($P = .05$) increased feed required per pound of gain.

Summaries of serum calcium and inorganic phosphorus are

given in Table 34 and of serum alkaline phosphatase and serum acid phosphatase in Table 35. The analysis of variance plan and breakdown of the statistical analysis for these determinations are given in Table 36. There were no significant treatment effects on serum calcium, inorganic phosphorus, or serum acid phosphatase. The addition of 100 mg of thyroprotein per pound of diet, however, increased serum alkaline phosphatase significantly ($P = .01$).

Three pigs died suddenly after being on experiment four to six weeks. All three were in Group 2, the portion of the experiment carried out during the winter, and all were receiving diets containing added thyroprotein. Postmortem examination revealed characteristics of hypervitaminosis A. Near the end of the 42-day experimental period gross symptoms of hypervitaminosis A were becoming apparent, especially in those pigs receiving thyroprotein. When blood samples were taken from the vena cava, it was apparent that the blood clotting mechanism was, in many cases, malfunctioning. Clotting time was obviously increased. It was also obvious that some, but not all, of the more severely affected pigs had very low hematocrit values, although this was not measured. Thyroprotein seemed to accelerate the development of hypervitaminosis A insofar as gross physical symptoms are concerned, but vitamin C had no apparent effect in either accelerating or retarding its development at the level used in the diet (100 mg/lb.).

Experiment 6421 - Vitamin A studies with swine

Objectives The purpose of this experiment was to determine if vitamins D, E, or K, alone or in combination, when fed with a diet containing 150,000 IU of added vitamin A per pound would have any influence on weight gain, feed per pound gain, serum calcium, inorganic phosphorus, alkaline phosphatase, or acid phosphatase.

Experimental Sixty-four pigs averaging 10.4 pounds and 16.9 days of age were allotted, four pigs per pen, and fed the experimental diets for a 30-day period. All diets contained 150,000 IU of added vitamin A per pound with either 500 or 50,000 IU of vitamin D, 0 or 15 mg of added vitamin K and 0 or 100 IU of vitamin E added per pound of diet. In this factorially arranged experiment each treatment was replicated twice. The experiment was conducted from May to June, 1964, in Unit D. Unit D provided concrete floored pens which were covered with wood shavings during the experiment. A 150-watt heat lamp provided supplemental heat during the first week when the temperature dropped below 65 degrees. Self-feeders and automatic waterers were provided in each pen. Weight gain and feed intake were recorded weekly. Serum calcium, inorganic phosphorus, alkaline phosphatase, and acid phosphatase were determined at the end of the 30-day experimental period. Composition of the basal ration appears in Table 4 in the Appendix.

Results and discussion Summaries of weight gain and feed required per pound of gain are presented in Figure 16 and Table 37. There were no significant differences in weight gain or feed per pound gain due to ration treatment.

Summaries of serum calcium and inorganic phosphorus are given in Table 39 and in Figure 17. Summaries of serum alkaline phosphatase and serum acid phosphatase are given in Table 40 and in Figure 18. The analysis of variance plan and the breakdown of the statistical analysis for these are given in Table 41. There was no significant ration treatment effect on serum calcium concentration. Pigs receiving 50,000 IU of vitamin D per pound of diet had significantly ($P = .05$) less serum inorganic phosphorus than those receiving 500 IU of vitamin D per pound. There was also a significant vitamin K by vitamin D interaction on serum inorganic phosphorus. The addition of 15 mg of vitamin K per pound of feed to diets containing 500 IU of vitamin D per pound decreased serum inorganic phosphorus, but the addition of this amount of vitamin K to diets containing 50,000 IU of vitamin D per pound resulted in higher levels of serum inorganic phosphorus in pigs in this experiment.

There were no significant ration treatment effects on serum acid phosphatase activity.

Serum alkaline phosphatase activity was increased significantly by the addition of 15 mg of vitamin K per pound of

Figure 16. Experiment 6421 - Effect of vitamins D, E, and K on weight gain (upper graph) and feed required per pound of gain (lower graph) with a diet containing 150,000 IU of added vitamin A per pound

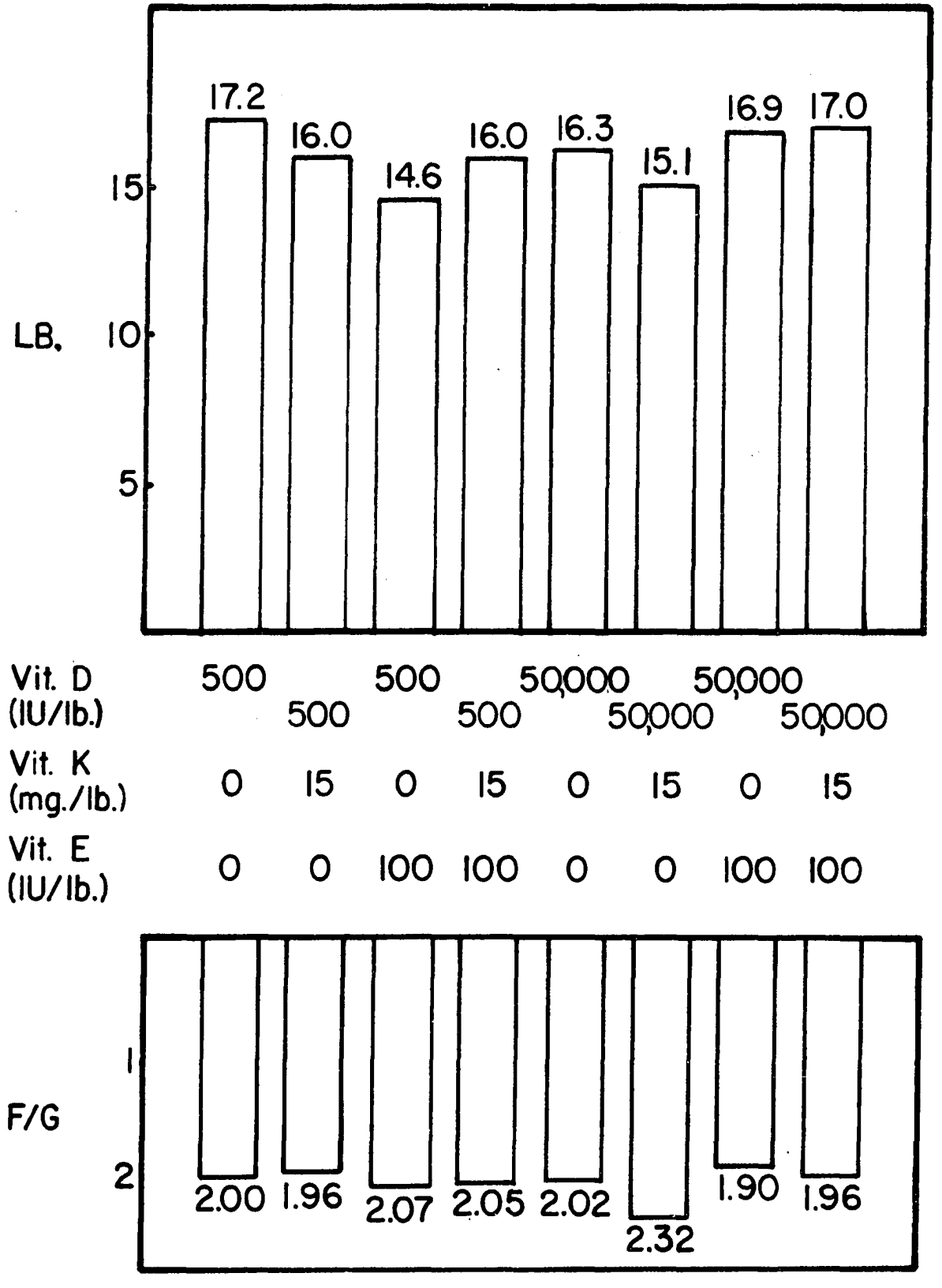
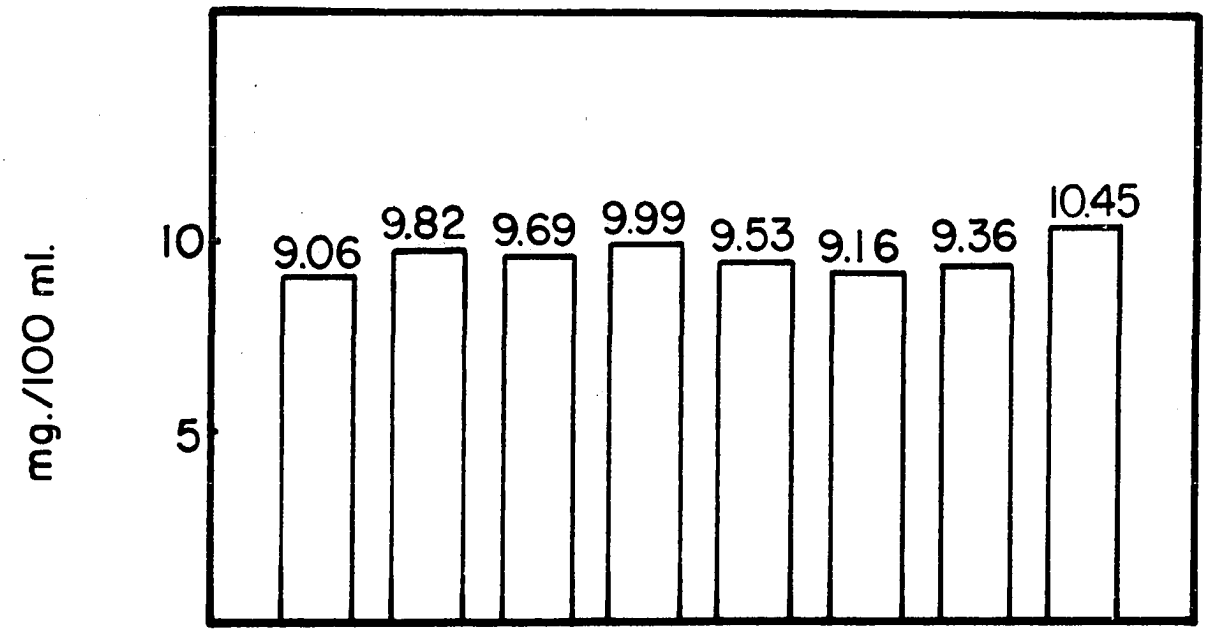

LB.
15
10
5
17.2
16.0
14.6
16.0
16.3
15.1
16.9
17.0
Vit. D (IU/lb.)
500 500 500 500 50,000 50,000 50,000 50,000
Vit. K (mg./lb.)
0 15 0 15 0 15 0 15
Vit. E (IU/lb.)
0 0 100 100 0 0 100 100
F/G
1
2
2.00 1.96 2.07 2.05 2.02 2.32 1.90 1.96

Figure 17. Experiment 6421 - Effect of vitamins D, E, and K on serum calcium (upper graph) and serum inorganic phosphorus (lower graph) with a diet containing 150,000 IU of added vitamin A per pound



Vit. D (IU/lb.)	500	500	500	500	50,000	50,000	50,000	50,000
Vit. K (mg./lb.)	0	15	0	15	0	15	0	15
Vit. E (mg./lb.)	0	0	100	100	0	0	100	100

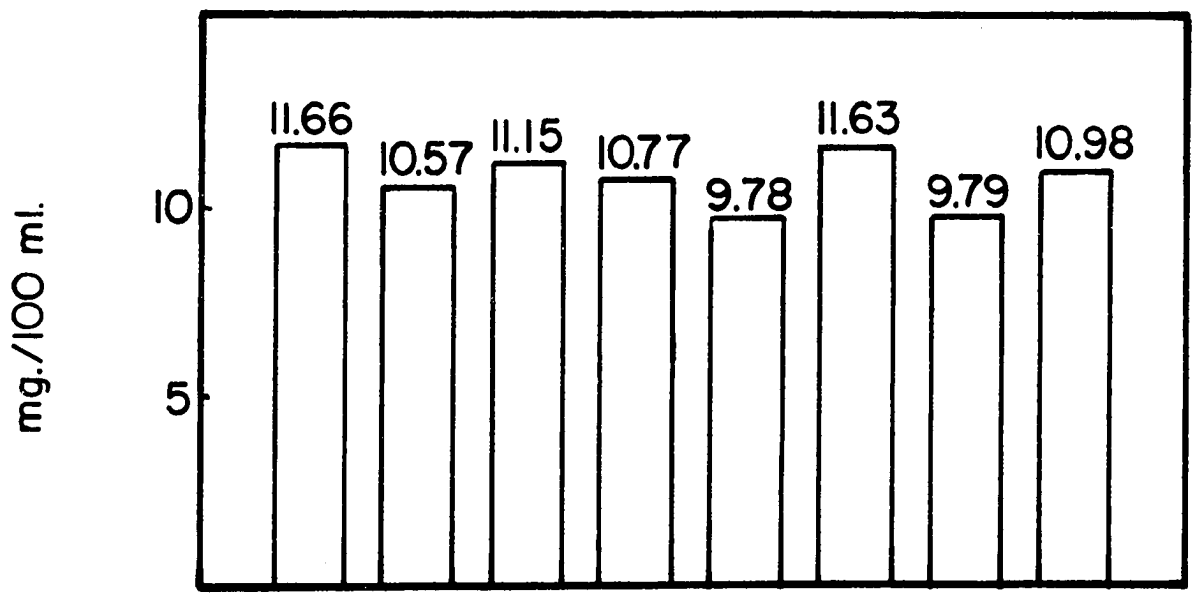
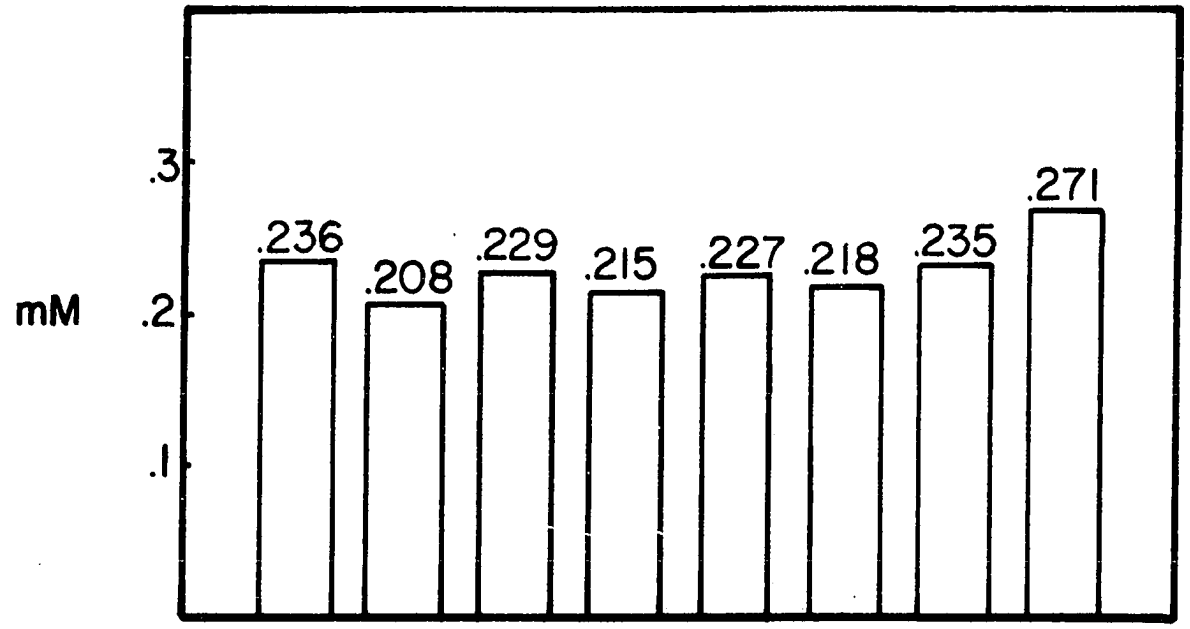
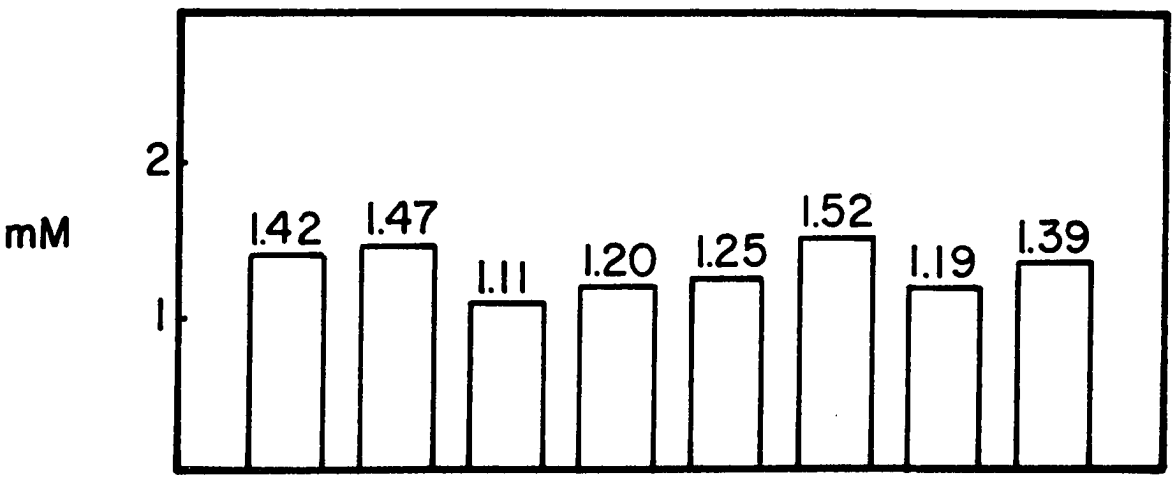


Figure 18. Experiment 6421 - Effect of vitamins D, E, and K on serum acid phosphatase activity (upper graph) and serum alkaline phosphatase activity (lower graph) with a diet containing 150,000 IU of vitamin A added per pound



Vit. D (IU/lb.)	500	500	500	500	50,000	50,000	50,000	50,000
Vit. K (mg./lb.)	0	15	0	15	0	15	0	15
Vit. E (IU/lb.)	0	0	100	100	0	0	100	100



diet, but the addition of 100 mg of vitamin E per pound of diet significantly depressed serum alkaline phosphatase activity. Both these effects were significant at P = .01.

It was expected that the high dietary level of vitamin A used in this experiment (150,000 IU per pound) would promptly produce symptoms of hypervitaminosis A since in several earlier experiments diets containing 100,000 IU per pound had produced hypervitaminosis A symptoms and even several deaths. Instead, the pigs in this experiment thrived on the high vitamin A feed.

The statistically significant effects of vitamin D on serum inorganic phosphorus concentrations and the vitamins D x K interaction pose an enigma which can not be satisfactorily explained on the basis of present day knowledge of the metabolic role of these vitamins. McChesney and Messer (1942) found that massive doses of vitamin D to rats resulted in increases in serum inorganic phosphorus (as well as serum calcium). Harrison and Harrison (1942) reported that massive doses of vitamin D to both normal and rachitic rats increased the concentration of phosphorus in the serum. They believed that the large dose of vitamin D increased the maximum ability of the kidney tubules to reabsorb phosphorus. This phenomenon may not result from prolonged administration of high levels of vitamin D such as the pigs in this experiment were subjected to.

The role of vitamin K in its apparent effect on serum inorganic phosphorus is no more clear than that of vitamin D. Vitamin K has been considered to play a role in electron transport and oxidative phosphorylation at least in microorganisms (Brodie, 1961) and recently evidence has been set forth which suggests that it may play a role in inducing RNA formation for the synthesis of blood clotting proteins (Olson, 1964). Confounding the role of vitamin K in the blood clotting mechanism and the effect of high dietary levels of vitamin A on prothrombin time is the report of Mellette and Leone (1960) who found that as they increased the level of tocopherol administered to rats, there resulted increased mortality and declining levels of prothrombin similar to that provoked by high levels of vitamin A. Whether the affects of vitamins D, E, and K on the blood serum phosphorus and alkaline phosphatase found in this experiment would also be found when the diet contained much less vitamin A is unknown. No conclusion concerning the effect of these vitamins on hypervitaminosis A can be drawn from this experiment.

Experiment 6409 - Vitamin A-carotene studies with swine

Objectives The purpose of this experiment was to ascertain if dehydrated alfalfa meal in the diet would precipitate hypervitaminosis A in pigs fed a diet containing 100,000 IU of added vitamin A per pound.

Experimental Sixty pigs averaging 9.9 pounds body weight and 15.7 days of age were allotted, four pigs per pen, and fed the experimental diets for a 42-day period. All diets contained 100,000 IU of added vitamin A per pound. Two levels of dehydrated alfalfa meal (5 and 10% of the diet) and two levels of beet pulp (5 and 10%) were added to the basal diet. The latter two treatments acted as comparison treatments from the standpoint of bulk in the diet. Each treatment was replicated three times.

The experiment was conducted during March and April, 1964, in unit D. Self-feeders and automatic waterers were provided in each pen. Feed and water were provided ad libitum. The composition of the basal ration is presented in Table 4 in the Appendix.

Criteria for evaluating the effect of alfalfa meal on a hypervitaminosis A diet were liveweight gains, feed per pound gain, and visual evaluation of toxicity symptoms. The experiment was six weeks in length and pig weights and feed consumption was measured at the end of one, three, and six weeks.

Results and discussion Summaries of weight gain and feed required per pound of gain are presented in Table 42 in the Appendix, and the analysis of variance plan and breakdown of the statistical analysis are given in Table 43.

There were no significant differences in average weight gain or in feed per pound gain due to ration treatment. Some

pigs on all treatments developed crooked legs, either in the front or rear quarters or both during the experiment, possibly as a result of the vitamin A in the diet, but no other indication of hypervitaminosis A was ever observed during the course of this six-week trial. There was no indication in this experiment that symptoms of hypervitaminosis A were potentiated by dehydrated alfalfa meal at 5 or 10% of the diet.

Experiment 6404 - Carotene and vitamin A studies with swine

Objectives The purpose of this experiment was to compare the effects of calculated equivalents of high dietary levels of beta-carotene and vitamin A in swine.

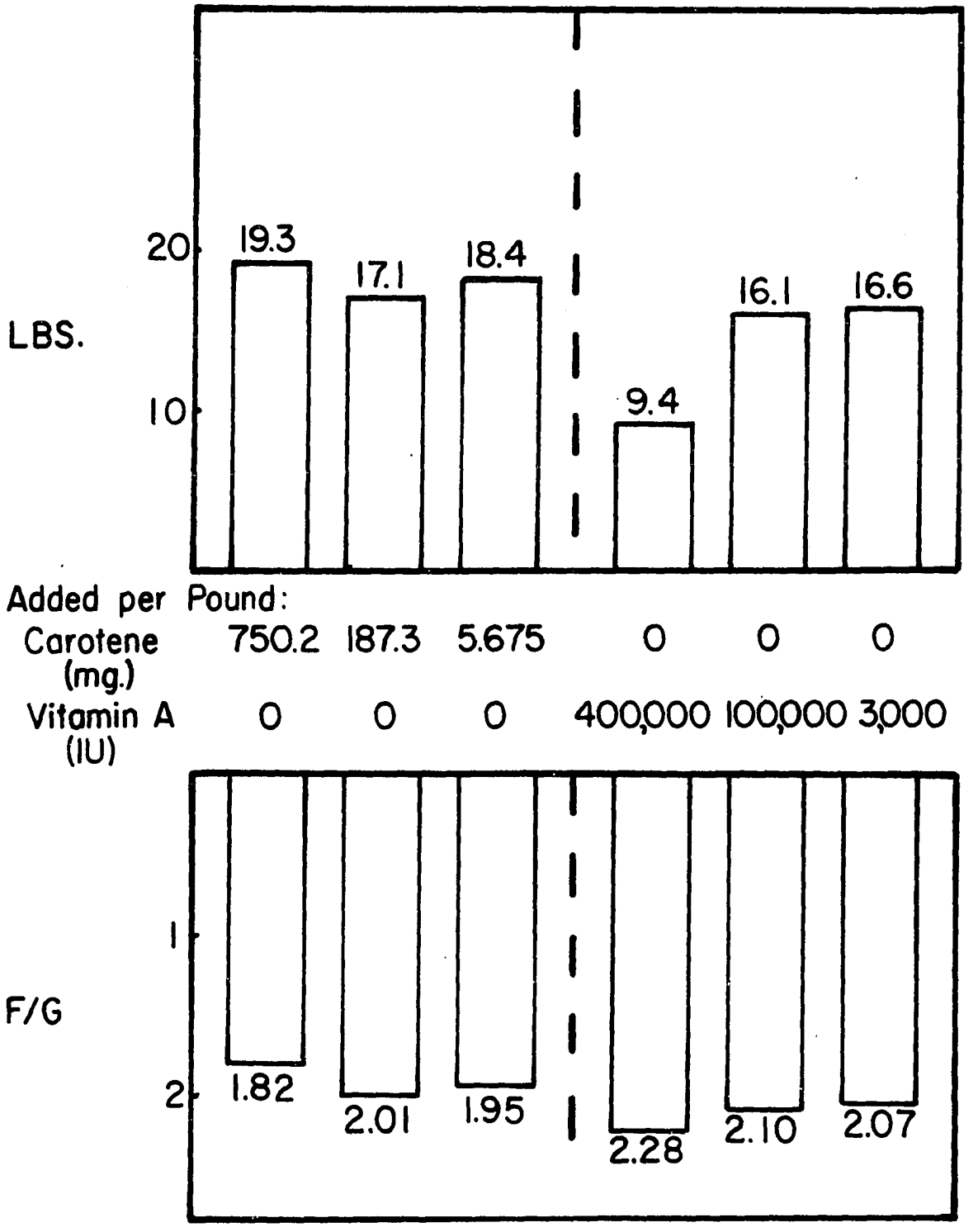
Experimental Thirty-six pigs averaging 9.2 pounds and 16.8 days of age were allotted, three pigs per pen, and fed the experimental rations for a 29-day period. The ration treatments were three levels of vitamin A (400,000, 100,000, and 3,000 IU) as vitamin A palmitate per pound of diet, and three levels of beta-carotene (750.2, 187.3, and 5.675 mg) added per pound of diet. The carotene levels were calculated to be potentially equivalent to those of vitamin A using one milligram of pure beta-carotene as equivalent to 533 IU of vitamin A. The carotene was supplied in beadlet form with a gelatin-carbohydrate carrier. There were two replications of each treatment.

The experiment was conducted during the months of February and March, 1964, in unit C which was previously described. Feed and water were supplied ad libitum. Weight gains and feed intake data were recorded weekly. Blood samples were drawn for analysis on day 26 of the experiment. The pigs were weighed off the experiment and metatarsal bones removed on day 29.

Weight gains, feed required per pound gain, blood serum calcium, inorganic phosphorus, alkaline phosphatase, and acid phosphatase were determined. Metatarsal bones were also fixed in alcoholic-formalin and decalcified in 5% formic acid. Histological preparations were made for microscopic examination using conventional techniques, stained with hematoxylin, and counterstained with eosin. Decalcified bone matrices also were hydrolized with 6N HCl in stoppered flasks for 36 hours at 100°C, and the resulting solution analyzed for hydroxyproline by the method described by Miyada and Tappel (1956).

Results and discussion Summaries of weight gain and feed required per pound of gain are given in Figure 19 and in Table 44. The analysis of variance plan and the breakdown of statistical analysis of these determinations are given in Table 45. Vitamin A, at the levels used, significantly ($P = .01$) depressed weight gains in pigs compared to those receiving a potential equivalent amount in the form of beta-

Figure 19. Experiment 6404 - Effect of calculated equivalent amounts of beta-carotene and vitamin A palmitate on weight gain (upper graph) and feed required per pound of gain (lower graph)


LBS.
20
10
19.3
17.1
18.4
9.4
16.1
16.6
Added per Pound:
Carotene (mg.)
750.2
187.3
5.675
0
0
0
Vitamin A (IU)
0
0
0
400,000
100,000
3,000
F/G
1
2
1.82
2.01
1.95
2.28
2.10
2.07

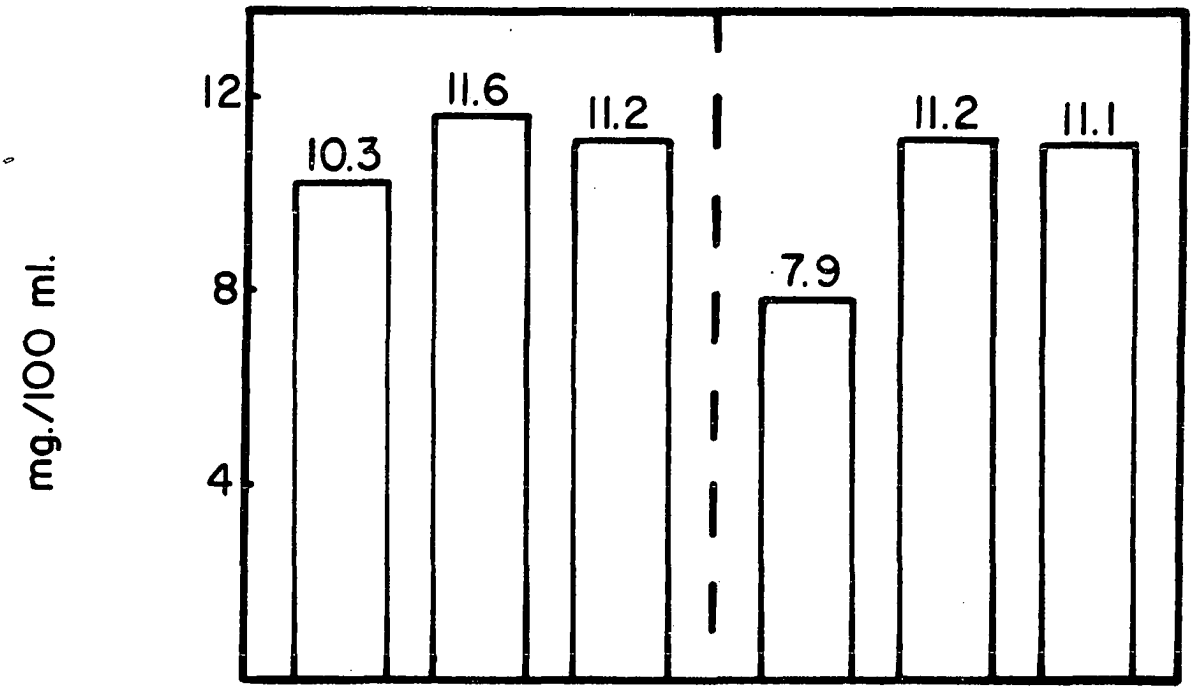
carotene. There was also a significant (P = .01) interaction between source and level of vitamin A on weight gain. There was no significant statistical difference between treatments in feed required per pound of gain, but those receiving the vitamin A palmitate were less efficient than those with beta-carotene as a source of added vitamin A.

Summaries of serum calcium and inorganic phosphorus are presented in Figure 20 and Table 45. The analysis of variance plan and breakdown of the statistical analysis for serum calcium and phosphorus as well as for weight gain and feed required per pound of gain are given in Table 46. Vitamin A palmitate significantly (P = .05) depressed serum calcium compared to this effect from carotene, but both sources of vitamin A activity significantly (P = .01) depressed serum calcium at the highest concentration used in the diet. No effect due to ration treatment on serum inorganic phosphorus was found.

Summaries of serum alkaline phosphatase and serum acid phosphatase are found in Figure 21 and in Table 47. That for bone alkaline phosphatase and bone acid phosphatase are presented in Figure 22 and in Table 48. The analysis of variance plan and breakdown of the statistical analysis of these determinations are given in Table 49.

Serum alkaline phosphatase was significantly depressed by vitamin A palmitate as compared with carotene. Analysis of the data revealed a significant (P = .01) depression in serum

Figure 20. Experiment 6404 - Effect of calculated equivalent amounts of beta-carotene and vitamin A palmitate on serum calcium (upper graph) and serum inorganic phosphorus (lower graph)



Added per Pound:						
Carotene (mg.)	750.2	187.3	5.675	0	0	0
Vitamin A (IU)	0	0	0	400,000	100,000	3,000

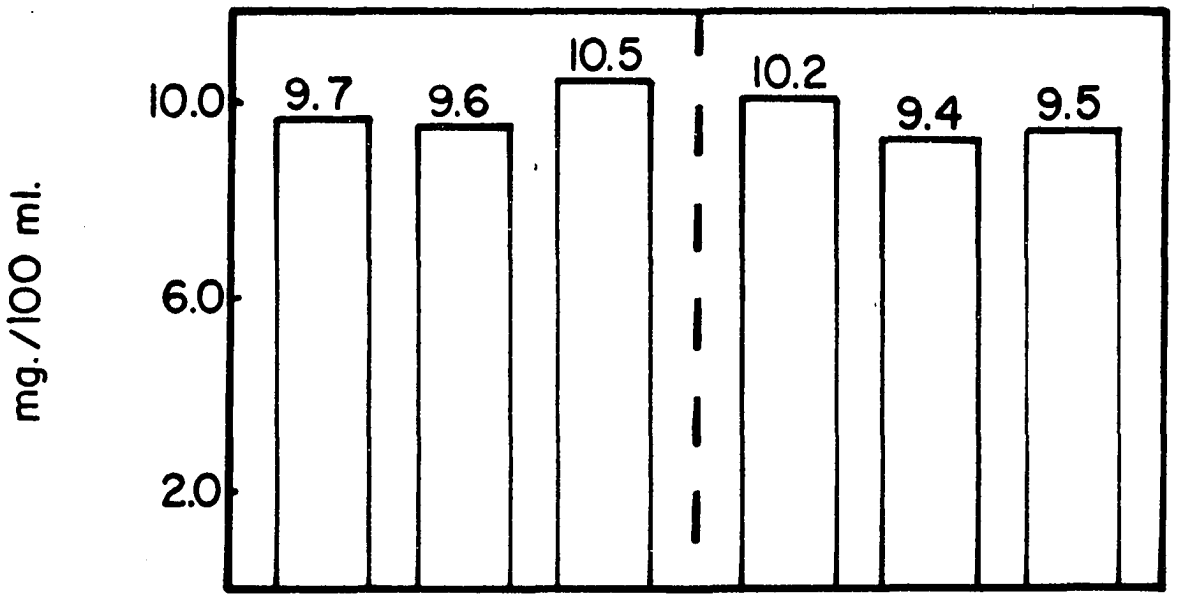


Figure 21. Experiment 6404 - Effect of calculated equivalent amounts of beta carotene and vitamin A palmitate on serum alkaline phosphatase activity (upper graph) and serum acid phosphatase activity (lower graph)

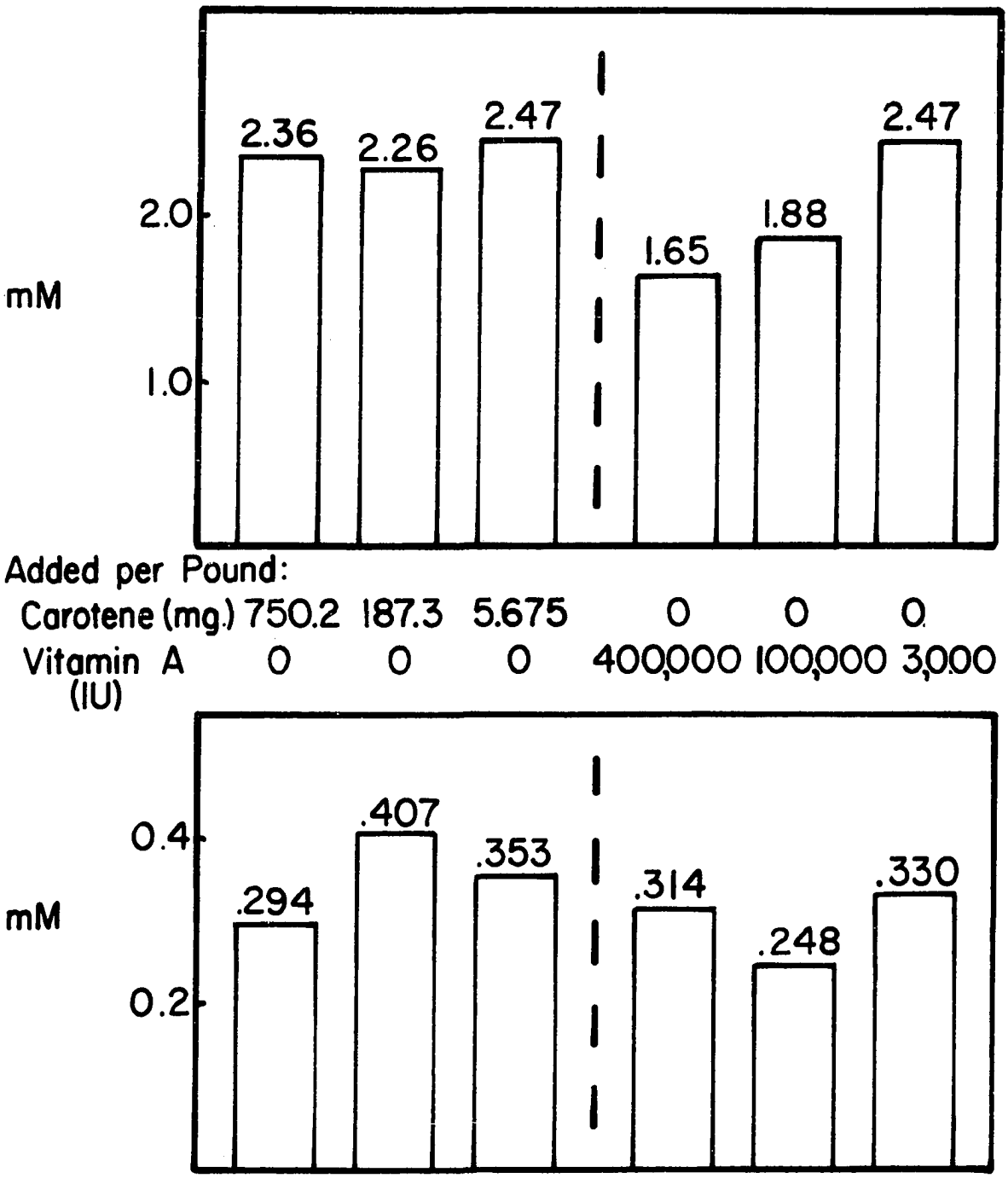
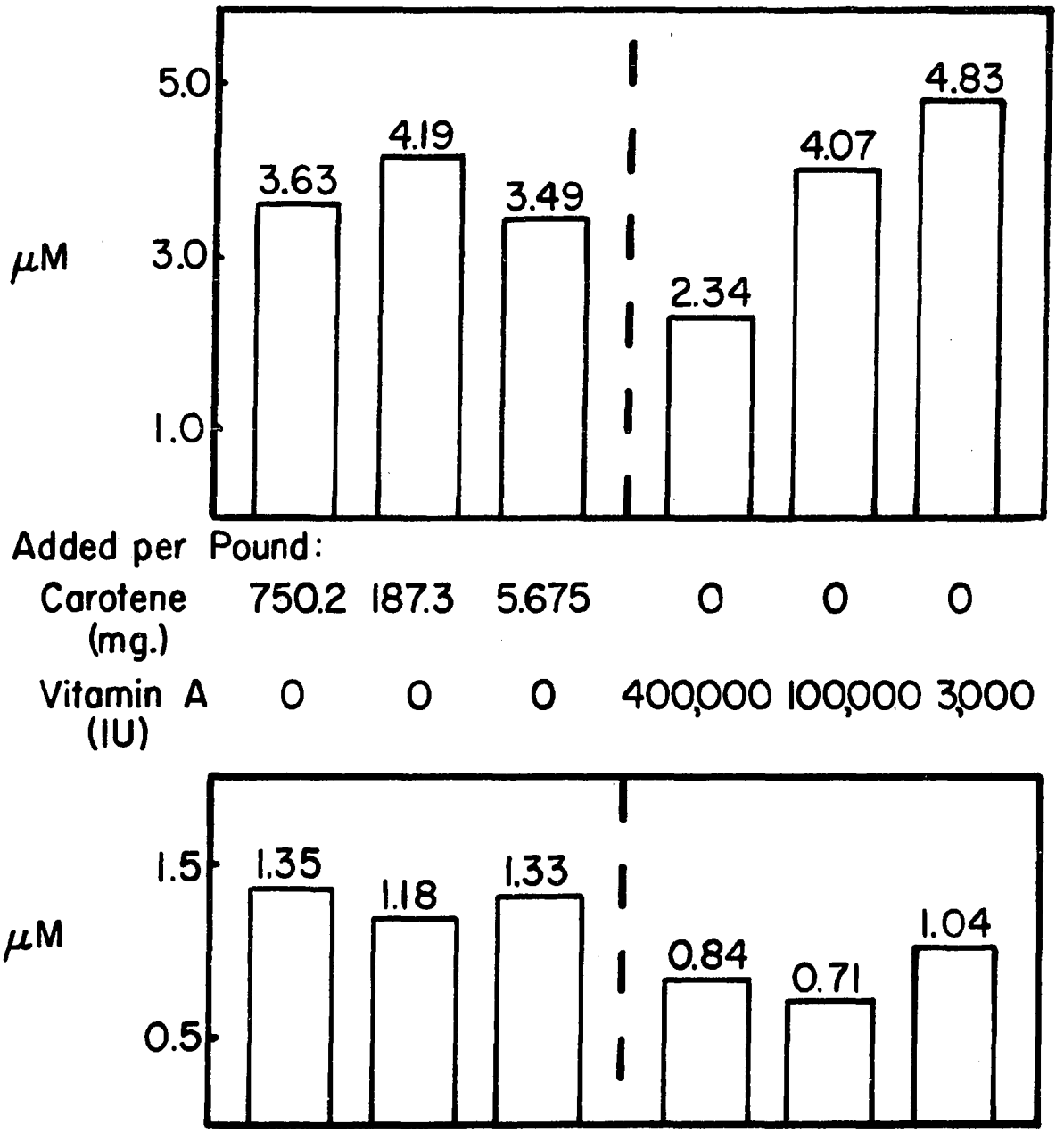

2.36
2.26
2.47
1.65
1.88
2.47
2.0
1.0
mM
Added per Pound:
Carotene (mg.) 750.2 187.3 5.675 0 0 0
Vitamin A (IU) 0 0 0 400,000 100,000 3,000
.294
.407
.353
.314
.248
.330
0.4
0.2
mM

Figure 22. Experiment 6404 - Effect of calculated equivalent amounts of beta-carotene and vitamin A palmitate on bone alkaline phosphatase activity (upper graph) and bone acid phosphatase activity (lower graph)


5.0
3.0
1.0
μM
3.63
4.19
3.49
2.34
4.07
4.83
Added per Pound:
Carotene (mg.) 750.2 187.3 5.675 0 0 0
Vitamin A (IU) 0 0 0 400,000 100,000 3,000
1.5
0.5
μM
1.35
1.18
1.33
0.84
0.71
1.04

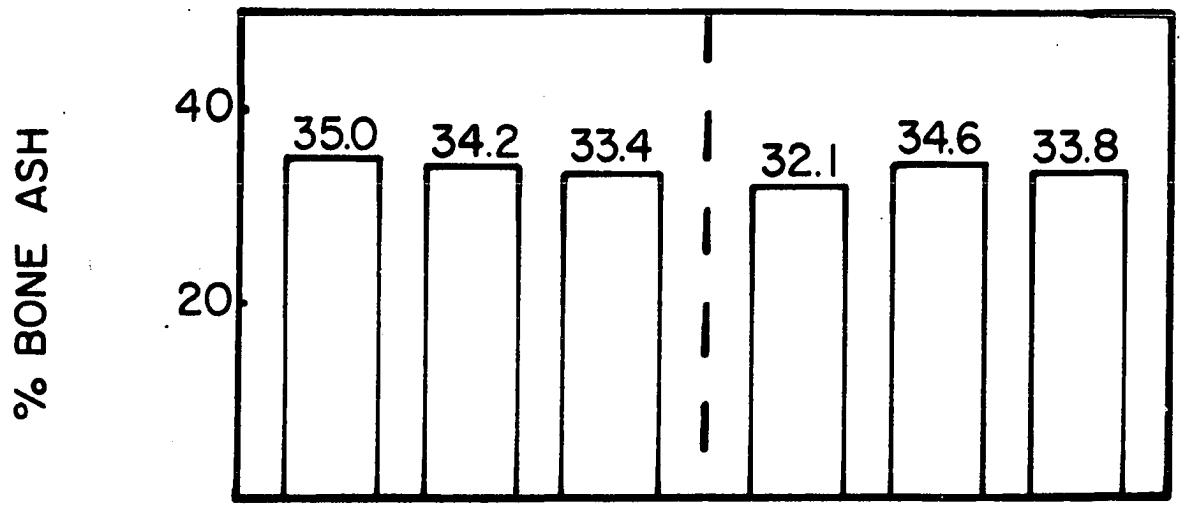
alkaline phosphatase as the level of vitamin A was increased. The serum alkaline phosphatase activity concentration in pigs receiving diets containing the added carotene was only slightly depressed with the higher levels of carotene. A significant ($P = .05$) source times level of vitamin interaction was found on serum alkaline phosphatase activity. There were no significant ration treatment effects on serum acid phosphatase activity.

Bones from pigs fed diets having a vitamin A potency of 400,000 IU per pound had significantly less ($P = .01$) alkaline phosphatase activity than those from pigs fed diets containing 100,000 IU of vitamin A potency. There were no significant differences in bone acid phosphatase activity due to ration treatment. Pigs fed vitamin A, however, had bones which, on the average, contained less bone alkaline phosphatase activity than those fed carotene as a source of vitamin A activity.

The summary of percent ash in the dry, fat-free bone is found in Table 44. The analysis of variance plan and breakdown of the statistical analysis are found in Table 49. The average bone ash values of pigs on each treatment are given in Figure 23. There were no significant differences due to ration treatment although percent ash increased at the highest dietary level of carotene compared to a decrease at the highest level of vitamin A palmitate.

Careful examination of the histological preparations of

Figure 23. Experiment 6404 - Effect of calculated equivalent amounts of beta-carotene and vitamin A palmitate on percent ash in bone



Added per Pound:						
Carotene (mg.)	750.2	187.3	5.675	0	0	0
Vitamin A (IU)	0	0	0	400,000	100,000	3,000

the metatarasal bones indicated that there were no distinct differences in the metaphyseal region of these bones. It was expected that cartilage columns would be found disorganized, there would be differences in the width of the epiphyseal cartilage plates, and other indications of hypervitaminosis A would be apparent. Either these bones are not influenced by hypervitaminosis A or these bones are more slowly affected than certain other organs or tissues of the body, for some of the bones examined were from pigs which had succumbed to hypervitaminosis A. Bones from approximately one-third of the animals were examined in this manner. It was decided to analyze the remaining decalcified bone matrices for hydroxyproline to determine if there was any indication that the collagen content had been affected by the sources and levels of vitamin A.

The summary of bone matrix hydroxyproline and the analysis of variance plan and breakdown of the statistical analysis for these determinations are given in Tables 50 and 51. The average percent hydroxyproline of the bone matrices are illustrated in Figure 24. There were no significant statistical differences due to ration treatment although it seems very suggestive that more hydroxyproline, and thus more collagen, was present in the matrices of bones of those pigs fed vitamin A palmitate than those fed carotene in the diet. Had more of the original matrices been available for analysis, a

Figure 24. Experiment 6404 - Effect of calculated equivalent amounts of beta-carotene and vitamin A palmitate on percent hydroxyproline in bone matrices

Added per Pound:						
Carotene (mg.)	750.2	187.3	5.675	0	0	0
Vitamin A (IU)	0	0	0	400,000	100,000	3,000

statistical difference may have been obtained.

Pigs fed the highest level of carotene exhibited some of the symptoms associated with hypervitaminosis A during the last week of the experiment. These included a decreased rate of gain, apparent stiffness in the leg joints, irritability, and greatly increased blood clotting time which became apparent when blood samples were taken. Two pigs on the highest carotene level died after approximately four weeks on the experimental diet. Examination of these pigs revealed massive abdominal hemorrhages.

Experiment 6428 - Vitamin A studies with swine

Objectives The purpose of this experiment was to determine the days required to produce hypervitaminosis A in young pigs fed diets containing different concentrations of vitamin A palmitate; to determine some of the possible effects of hypervitaminosis A; and to observe what effects a high dietary level of beta-carotene would have on pigs.

Experimental Twenty-four pigs averaging 10.2 pounds and 14.5 days of age were weaned and allotted to individual metal pens with wire mesh floors in unit E. The treatments were 0, 100,000, 200,000, 300,000, and 400,000 IU of vitamin A palmitate and 938 mg of carotene (calculated equivalent to 500,000 IU) of vitamin A per pound of diet. The experiment was conducted during July-September, 1964. Feed and water

were provided ad libitum. Weight gain and feed intake were recorded weekly. After four weeks, hemoglobin, hematocrit, and plasma hydroxyproline were determined. The composition of the basal ration is presented in Table 4 in the Appendix.

Hemoglobin levels were determined by the acid hematin method. The hematocrit was determined with calibrated centrifuge tubes. Blood was centrifuged and the plasma deproteinated with a 3% solution of 5-sulfosalicylic acid and hydroxyproline in the resulting deproteinated plasma determined in the manner described by Miyada and Tappel (1956).

Results and discussion Most of the pigs in this experiment were slow to learn to eat, consequently after two weeks, seven of the original 24 animals had to be replaced. There was no treatment effect on refusal to eat. The average starting weights and age of the 24 pigs ultimately used in this experiment were 10.3 pounds and 16.7 days. The replacement pigs were fed the basal ration together as a group on the floor for three days prior to allotment to the individual pens and dietary treatments, consequently they began to eat the experimental diets without hesitation.

Symptoms of hypervitaminosis A appeared in certain replacement pigs after 10 days and in certain of the remaining original pigs after 24 days on the experimental treatments. It appeared that a major factor in the time required for symptoms of hypervitaminosis A to develop in weanling pigs,

as one would expect, was the time required to learn to eat feed. In this experiment the replacement pigs which had learned to eat before allotment to the individual pens were eating nearly as well after 10 days as the original pigs were after four weeks, and in some of these, symptoms of hypervitaminosis A were apparent. Blood samples for hematocrit, hemoglobin, and free plasma hydroxyproline analysis were taken at this time.

The summary of weight gain at four weeks is given in Table 52, and the analysis of variance plan and breakdown of the statistical analysis for weight gain are given in Table 53. Summaries of plasma hydroxyproline, blood hemoglobin, and hematocrit are given in Table 54, and the analysis of variance plan and breakdown of the statistical analysis of these are given in Table 55. In the statistical analysis of this data, the carotene treatment was considered equivalent to 500,000 IU of added vitamin A per pound. Four-week weight gains were determined after each pig had been on his experimental treatment for four weeks, or if removed earlier because of severe symptoms of hypervitaminosis A or death, the weight at that time was used.

There was a significant ($P = .01$) linear decrease in weight gain as the dietary vitamin A level increased. The average weight gain of pigs on the carotene treatment at four weeks was comparable to that of pigs receiving between 200,000

and 300,000 IU of vitamin A palmitate per pound.

There was a significant (P = .01) linear increase in plasma hydroxyproline concentration with the increasing vitamin A potency of the diet. The pigs on the diet containing the carotene had an average plasma hydroxyproline level in the plasma nearly as great as pigs fed diets containing 400,000 IU of added vitamin A palmitate per pound of diet.

There was a significant (P = .01) quartic response to treatment on hemoglobin and hematocrit. The biological reason for this is obscure.

At this time, four weeks after the original start of the trial, the pigs were transferred to unit D and confined to allotted pens according to treatment to determine the time required to produce symptoms of hypervitaminosis A in the pigs remaining. The results are summarized in Table 3.

One pig receiving the high level of carotene in the diet died suddenly after 24 days on the experimental diet. Postmortem examination revealed a massive hematocyst between the right scapula and thoracic wall, and a subcutaneous hematocyst on a leg joint. Death was due to exsanguination. These findings are highly suggestive of vitamin A (carotene) toxicity.

Pigs fed diets containing 100,000 IU of vitamin A palmitate per pound did not at any time appear to be adversely affected. The weight gain of these pigs at the end of eight weeks was greater than those on any other treatment including

Table 3. Experiment 6428 - Dose-time relationships in producing symptoms of hypervitaminosis A

Treatment	Added per pound of diet	Days to first pig with symptoms	Number of pigs affected	Average days for all pigs
1	0	--	--	--
2	100,000[a]	--	--	--[b]
3	200,000[a]	28	4	43
4	300,000[a]	32	4	32
5	400,000[a]	10	4	17.5
6	500,000[c]	24	1[d]	--[b]

[a]IU of vitamin A palmitate.

[b]Terminated after eight weeks.

[c]Beta-carotene (938 mg per pound); calculated equivalent to 500,000 IU of vitamin A based on 1 mg = 533 IU.

[d]Pig died suddenly; postmortem examination revealed symptoms characteristic of hypervitaminosis A.

the basal diet which contained no added vitamin A.

Earlier experiments had indicated that diets containing 100,000 IU of vitamin A per pound would prove toxic to pigs in four to six weeks. Observations made in earlier experiments and supported by comments in published reports for other species indicate that older animals are less susceptible to hypervitaminosis A than younger ones. Possibly the slow initial growth rate of these pigs, due to early reluctance to

eat, when penned individually after weaning, was an important factor in this regard; when feed consumption and growth rate increased later, possibly the pigs were old enough to be resistant to any affect possible at this level of feeding.

Only one pig was overcome by carotene feeding. This pig showed evidence of disorders similar to those found with vitamin A toxicity. Early in the experiment the high level of carotene seemed to promote various degrees of diarrhea in contrast to the other diets containing vitamin A. At the end of the eight-week period when the experiment was terminated, the average weight gains of the pigs on the carotene diet were similar to those on the control diet. Except for indirect evidence in the pigs that died, there was no indication of any interference in blood clotting mechanisms during the course of this experiment. The erratic values found in hemoglobin and hematocrit determinations suggest individual pig variation in response to hypervitaminosis A, for unquestionably some of the animals were severely affected by the vitamin yet there was no consistent response in these blood values.

GENERAL DISCUSSION

Reports of hypervitaminosis A are largely confined to experimental studies on the subject; however, examples of accidental or incidental hypervitaminosis A are not uncommon and seem to be occurring with increasing frequency. The chief cause in humans is the compulsive self-administration of vitamin preparations. Hypervitaminosis A in farm animals probably only occurs accidentally as a result of carelessness or ineptness in formulating rations or mixing feed or through improper labeling, handling, or use of highly concentrated vitamin premixes. The work in Australia, New Zealand, and parts of Europe culminating in papers of Grant and O'Hara (1957) and Weits (1960), for example, indicate that vitamin A or one of its precursors, at relatively high but non-toxic levels in the diet, can exert a detrimental influence on the health of animals, at least under certain conditions.

Over the years, experimental studies of hypervitaminosis A have been carried out with a variety of animal species and for several purposes. Some experiments have been conducted to estimate safe therapeutic levels to use in treating patients depleted or deficient in this vitamin. Others have been conducted simply to characterize the toxicity in one or more species, while others involve hypervitaminosis A in vitamin-disease interrelationships. Hypervitaminosis A

studies have been used in an attempt to gain insight to the normal metabolic role of vitamin A and to search for interrelationships between this and other vitamins.

The varied purposes of the investigations, species and ages of the animals used, source, potency, method of administration, duration of the application of the experimental treatment, and other factors all have contributed to the many often conflicting and confusing reports on hypervitaminosis A. For these reasons it is impossible to extrapolate with complete confidence from one species to another regarding hypervitaminosis A.

It was observed in experiments reported in this dissertation that the order of appearance or development of gross symptoms of hypervitaminosis A in the pig may vary depending on the level of the vitamin in the feed consumed (dose). Some pigs fed diets containing 400,000 or 500,000 IU of added vitamin A per pound often exhibited no obvious signs of malaise or toxicity yet died suddenly due to massive internal hemorrhages. The condition of other pigs on these treatments would generally rapidly deteriorate and, unless immediately removed from these rations at the time of the first indication of illness, would often be unable to subsequently develop normally.

Animals subjected to lower dietary levels of vitamin A usually developed symptoms of hypervitaminosis A gradually. Daily feed intake declined with the onset of gross symptoms

of hypervitaminosis A, but this did not appear to retard or prevent further development of the hypervitaminosis A condition. When fed the same ration containing no added vitamin A, further deterioration of condition was usually arrested, and improvement became apparent in one to three weeks.

Feed required per pound of gain increased significantly in pigs displaying symptoms of hypervitaminosis A, but not in pigs fed high levels (100,000 or 150,000 IU per pound of feed) unless gross physical symptoms of hypervitaminosis A appeared.

In these experiments serum calcium was generally insignificantly affected by high dietary levels of vitamin A. Four hundred thousand units of vitamin A per pound of diet significantly depressed serum calcium in Experiment 6404. One hundred thousand units of vitamin A per pound of diet slightly, but significantly, increased serum calcium in Experiment 6327, while in the other four experiments where serum calcium was measured the concentration was not altered.

Rodahl (1950) observed no significant changes in serum calcium in young rats due to hypervitaminosis A, but Dull et al. (1961) found that high level doses of vitamin A to adult humans would depress serum calcium.

In Experiment 6327 increased concentrations of vitamin D in the feed resulted in increased serum calcium, but this effect of vitamin D did not occur when 100,000 IU of vitamin A was added per pound of feed. Earlier Clark and Bassett

(1962) with rats had found that large doses of vitamin A prevented or reduced the effects of vitamin D toxicity.

In Experiment 1179 the addition of 500, 5,000, and 50,000 IU of vitamin A per pound of diet was accompanied by a corresponding increase in serum inorganic phosphorus and the addition of 100,000 IU of vitamin A to the diet in Experiment 6327 resulted in a slight, but statistically significant increase in the inorganic phosphorus in blood serum. Other experiments where 100,000 or 150,000 IU were added per pound of diet resulted in no significant differences in the inorganic phosphorus content of the serum of the pigs.

Five hundred thousand IU (Experiment 1179) but not 400,000 IU of vitamin A added per pound of diet (Experiment 6404) caused a decrease in serum inorganic phosphorus. Failure for the effect of these two high dosage diets to provide the same type response in serum phosphorus in pigs definitely afflicted with hypervitaminosis A, and the usual inability to show a statistical treatment response at lower, though sometimes toxic levels (100,000 IU per pound of feed), forces the conclusion that hypervitaminosis A does not affect serum inorganic phosphorus concentrations of the young pig. The same conclusion was drawn by Rodahl (1950) with rats. The relationship between vitamins D and K in a diet containing 150,000 IU of vitamin A per pound in affecting serum phosphorus levels remain inexplicable.

The serum alkaline phosphatase activity in pigs in Experiment 6421 was depressed by vitamin E and increased by vitamin K additions to the diet. In Experiment 6404 the pigs fed diets high in carotene had a greater serum concentration of alkaline phosphatase activity than those fed calculated equivalent amounts of vitamin A palmitate. Increased levels of both vitamin A and carotene in this experiment resulted in lower concentrations of phosphatase activity in the serum. In general, the results of other experiments (1179 and 6327) also indicate that a decreased concentration of serum alkaline phosphatase activity was likely to occur with high (100,000 IU or more) vitamin A per pound of diet.

On the other hand, the addition of 100 mg of thyroprotein (iodinated casein) per pound of diet increased serum alkaline phosphatase activity in Experiment 6318. Sadhu and Brody (1947) and Viel et al. (1961) among others have found that hypervitaminosis A will cause a reduced basal metabolic rate in animals. In this experiment (6318) there was an increase in the amount of feed required per pound of gain, and the increased serum alkaline phosphatase may be a reflection of intestinal phosphatases for it has been shown that feed intake can affect the content of alkaline phosphatase activity in intestinal tissue (Madsen and Tuba, 1952). In Experiments 6327 and 6404 there were no differences in feed intake per pound of gain or in weight gains due to level of vitamin A

in the diet and yet serum alkaline phosphatase activity was depressed with increased dietary vitamin A.

Treatments containing 100,000 IU or more vitamin A per pound of diet also depressed the alkaline phosphatase activity content of metatarsal bones in experiments where this was measured (Experiments 1179, 6327, and 6404). Other things being equal, it is thought that bone contributes heavily to serum alkaline phosphatase levels (Madsen and Tuba, 1952), at least in young animals. Ludwig (1953) found that hypervitaminosis A resulted in increases in the alkaline phosphatase at epiphyseal junctions of some bones and decreases in the amount of this enzyme in other bones. Wolbach (1946) considered excess vitamin A to accelerate the maturation of bone. It is known that the alkaline phosphatase content of bones decreases with age or maturity. Dickerson (1962) found that the fat content increases and the water content of bone decreases with age. The decreased alkaline phosphatase of bones (Experiments 1179, 6327, and 6404) with high levels of vitamin A and increased ether extract and decreased water content of bones as the log dose of vitamin A increased (Experiment 1179) tend to support the idea that hypervitaminosis A accelerates bone maturation.

In Experiment 6404 it was found that pigs receiving vitamin A palmitate had metatarsal bones with matrices containing a greater percent hydroxyproline than pigs fed similar amounts

of carotene. This could also suggest a "maturation" effect due to vitamin A palmitate for Dickerson (1962) found that collagen as a percent of fat-free bone (pig humerus) increased with age in normal pigs up to about 56 days of age. This increase (Dickerson, 1962) was ascribed to a decrease in water content. After about eight weeks of age, the collagen concentration decreased and this was believed due to a relative increase in mineralization. In Experiment 1179 the average percent ash of dry, fat-free bone decreased as the log dose of vitamin A increased although the effect was not statistically significant. Both bone ash percent calcium and phosphorus increased as the log dose increased, and while only the latter effect was statistically significant, it may indicate increased mineralization taking place. Observation of pigs six months after severe hypervitaminosis A indicated that the long bones of the limbs had stopped growing in length at an early age (Experiment 1179).

Klein et al. (1962) considered increased urinary excretion of hydroxyproline as indicative of increased formation of soluble bone collagen. A major part of his argument was based on the fact that there is both a rapid increase in bone matrix formation (collagen formation) and increased urinary excretion of hydroxyproline when vitamin C is administered to young scorbutic animals. Conversely, Bastomsky and Dull (1962) considered increased urinary excretion of hydroxypro-

line to indicate bone destruction for they found that triiodothyronine administered to rats resulted in both bone rarifaction and increased urinary excretion of hydroxyproline.

In Experiment 6428 a significant linear increase in free plasma hydroxyproline accompanied higher dietary levels of vitamin A. This coupled with the greater percent hydroxyproline concentration in the bone matrices in pigs fed high levels of vitamin A in Experiment 6404 seems to support the view of Klein et al. (1962). On the other hand, one could consider that the higher hydroxyproline concentrations in bone of pigs fed high levels of vitamin A as a result of loss of ground substance, elastin, or a change in the composition of the bone collagen rather than an increase in the proportion of collagen. The last possibility seems unlikely in view of the work of Eastoe (1955) and others that indicates the amino acid composition of bone matrix collagen is quite stable. The increase in plasma hydroxyproline could be due to breakdown of connective tissue collagen of tissues other than bone. The widespread hemorrhages which occur in hypervitaminosis A may be due to failure of the blood to clot promptly (hypoprothrombinemia), but the massive and fatal hemorrhages so frequently encountered must surely be accomplished by a certain amount of structural breakdown or disintegration of the tissue involved. It is perhaps significant in this regard that in Experiment 6428 the plasma free hydroxyproline concentration

of pigs fed the carotene was as high as those fed high levels of vitamin A palmitate. Further, the metatarsal bone matrices of the pigs fed carotene (Experiment 6404) contained the same average hydroxyproline concentration regardless of level of carotene fed, yet some pigs on the diets containing high levels of carotene in both these experiments did die of massive internal hemorrhage similar to that found in pigs fed toxic amounts of vitamin A palmitate.

The only indications that high dietary levels of beta-carotene are detrimental to the health status of pigs were occasional evidence of a malfunctioning blood clotting mechanism and the sudden death of several pigs due to massive internal hemorrhage similar to that found when toxicity from vitamin A palmitate was the cause of death.

Decreased hematocrit and hemoglobin concentration in the blood were observed occasionally in pigs severely affected with hypervitaminosis A in these experiments, but were not encountered so frequently as was the condition of prolonged blood clotting time. These three blood related changes have been reported as characteristic of hypervitaminosis A in other species, but there is apparently considerable individual animal variation in development of these particular symptoms.

Ershoff et al. (1957) found that diets containing 5 and 10% alfalfa meal potentiated symptoms of hypervitaminosis A in rats. His rat diets contained 2.5 million units of vitamin

A palmitate per kilogram, a vitamin A concentration which undoubtedly would produce death in 100% of pigs. In Experiment 6409 there was no indication that the addition of 5 or 10% dehydrated alfalfa meal to the diet would potentiate the activity of 100,000 IU of vitamin A palmitate per pound of diet to produce symptoms of hypervitaminosis A in pigs.

One hundred thousand IU per pound of diet seemed to be a point at which young pigs may sometimes develop hypervitaminosis A in these experiments. The controlling factor(s) determining whether or not symptoms of hypervitaminosis A do develop when the diet contains 100,000 IU of vitamin A per pound are unclear. Lewis (1954) obtained toxicity in pigs by eight weeks with a diet containing less than 70,000 IU per pound, and Frape claimed to have observed weight gain depression in pigs fed about 12,000 IU of vitamin A per pound of diet. In Experiments 6311, 6318, and 6404 vitamin A palmitate added at the rate of 100,000 IU per pound to the diet caused definite symptoms of hypervitaminosis A, including some deaths, in four to six weeks. The addition of 100,000 IU per pound of diet in Experiment 6409 and 150,000 IU per pound of diet in Experiment 6421 failed to produce overt symptoms of hypervitaminosis A during the experimental period. Eight weeks on a diet containing 100,000 IU of added vitamin A palmitate per pound failed to produce any evidence of toxicity in Experiment 6428, while in the same experiment 400,000,

300,000 and 200,000 IU per pound of diet produced definite hypervitaminosis A on the average in 17.5, 32, and 43 days, respectively.

SUMMARY

Eight experiments were conducted to study the effect of hypervitaminosis A in the young pig. It was observed that younger and/or smaller pigs were more susceptible to hypervitaminosis A than older or larger pigs. Diets containing 500,000 IU of vitamin A palmitate per pound produced gross symptoms of hypervitaminosis A by two weeks time. Diets containing 400,000, 300,000, and 200,000 IU of vitamin A palmitate per pound produced symptoms, on the average, in pigs in 17.5, 32, and 43 days, respectively. The addition of 100,000 units of the vitamin per pound of diet in some experiments produced hypervitaminosis A in four to six weeks; in other experiments no toxicity was noted.

Extremely high dietary levels of vitamin A generally resulted in sudden death due to massive internal hemorrhage. Lesser amounts of vitamin A (100,000 or 200,000 IU per pound of diet) produced symptoms of hypervitaminosis A gradually, with the first indication of the onset of the condition often being delayed blood clotting time. Then a decline in physical condition, a decreased rate of gain, a decreased feed intake, and an increased feed required per pound of gain followed. Reluctance to move about the pen and increased irritability were followed by loss of control of rear legs, and later, inability to rise at all. Tremors were not uncommon at this

time. Leg joints often became swollen, and numerous subcutaneous hemorrhages developed, especially on the legs, but also on the lower abdomen. The skin became dry and scaly; the hair coat roughened. At times there was considerable lacrimation from the eyes of the pigs most seriously affected. The urine and/or feces occasionally contained blood.

Internal hemorrhages often occurred at limb joints, in the kidney, and in the intestinal tract. Subpericardial hemorrhages also occurred. Death was ascribed to internal hemorrhage in all cases.

Costochondral junctions have been found enlarged and appeared decalcified; cartilage cell columns were disorganized. Metatarsal bones did not seem to be affected histologically. Early and permanent cessation of longitudinal growth of long bones of the limbs occurred.

Hypervitaminosis A was found to have no consistent effect on serum calcium, inorganic phosphorus, or acid phosphatase. Free plasma hydroxyproline increased as dietary vitamin A increased. Serum and bone alkaline phosphatase activity decreased with hypervitaminosis A, and increased with thyroprotein added to the diet. Metatarsal bone calcium, phosphorus, and acid phosphatase were not consistently affected by hypervitaminosis A, but high dietary levels of the vitamin resulted in increased bone matrix hydroxyproline.

Vitamin C (100 mg per pound of diet) had no apparent

effect, but 100 mg of thyroprotein per pound of diet accelerated the appearance of symptoms of hypervitaminosis A in pigs fed diets containing 100,000 IU per pound.

In a diet containing 150,000 IU of added vitamin A per pound, vitamin D (50,000 IU/lb.), vitamin E (100 mg/lb.), and vitamin K (15 mg/lb.) had no effect on the gross appearance of the pigs after 30 days, but the high vitamin D level depressed serum inorganic phosphorus, vitamin E depressed serum alkaline phosphatase activity, and vitamin K increased serum alkaline phosphatase activity.

Blood hemoglobin and hematocrit seemed to be depressed only during the latter, critical stages of hypervitaminosis A, if at all.

Diets containing 5 or 10% dehydrated alfalfa meal did not potentiate symptoms of hypervitaminosis A in pigs fed 100,000 IU of vitamin A palmitate per pound of diet. High dietary levels of carotene added to diets (938 or 750.2 mg per pound) sometimes caused increased blood clotting times and occasionally death in pigs which at necropsy were found to have succumbed to massive internal hemorrhages similar to those found in deaths caused by excess vitamin A palmitate.

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APPENDIX

Table 4. Composition of basal rations

Ingredient	Experiment							
	1179	6311	6318	6327	6404	6409	6421	6428
Ground yellow corn	71.95	67.40	67.40	67.40	67.40	67.39	67.39	67.39
Soybean meal (50% protein)	23.25	27.85	27.85	27.85	27.90	27.90	27.90	27.90
Iodized salt	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Calcium carbonate (38% calcium)	0.80	0.85	0.85	0.85	0.85	0.84	0.84	0.84
Dicalcium phosphate (26% calcium) (18% phosphorus)	1.30	1.20	1.20	1.20	1.15	1.17	1.17	1.17
Vitamin premix[a]	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Trace mineral premix[b]	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

[a]The composition is given in Table 6.

[b]The composition is given in Table 7.

Table 5. Calculated analysis of basal rations

Item		1179	6311	6318	6327	6404	6409	6421	6428
Protein	percent	18	20	20	20	20	20	20	20
Fat	percent	2.83	2.75	2.75	2.75	2.75	2.75	2.75	2.76
Fiber	percent	2.52	2.56	2.56	2.56	2.56	2.56	2.56	2.57
Calcium	percent	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70
Phosphorus	percent	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55
Vitamin A	IU/lb.	730	686	100686	686	3688	100686	150687	691
Vitamin D_2	IU/lb.	500	500	500	0	500	500	500	500
Riboflavin	mg/lb.	5.4	4.7	4.7	4.7	8.7	8.7	8.7	8.7
Pantothenic acid	mg/lb.	12.7	11.3	11.3	11.3	19.3	19.3	19.3	19.3
Niacin	mg/lb.	31.0	27.4	27.4	27.4	45.4	45.4	45.4	45.4
Choline	mg/lb.	448	500	500	500	540	540	540	540
Vitamin B_{12}	mcg/lb.	20	20	20	20	20	20	20	20

Table 6. Amounts of vitamins and antibiotics added per pound of basal ration

Ingredient		Experiment							
		1179	6311	6318	6327	6404	6409	6421	6428
Vitamin A	IU	0	0	100000	0	3000	100000	150000	0
Vitamin D_2	IU	497	500	500	0	500	500	500	500
Riboflavin	mg	4	4	4	4	8	8	8	8
Calcium pantothenate	mg	10	8	8	8	16	16	16	16
Niacin	mg	22	18	18	18	36	36	36	36
Choline	mg	0	0	0	0	40	40	40	40
Vitamin B_{12}	mcg	20	20	20	20	20	20	20	20
Chlortetracycline	mg	100	50	50	50	50	50	50	50

Table 7. Trace mineral premix (35-C-41)

Element	Percent in premix	Parts per million contributed to ration by 0.20%
Iron	7.0	140.0
Copper	0.475	9.5
Cobalt	0.166	3.3
Zinc	8.10	162.0
Manganese	5.68	113.6
Calcium	5.28	--
Potassium	0.750	15.0

Table 8. Experiment 1179 - Summary of weight gain, feed per pound gain, and average daily feed consumption

Added vitamin A (IU/lb.)	0	500	5,000	50,000	500,000
Replication			Total gain (lb.)		
1	28.83	33.17	29.50	30.67	10.25
2	25.67	28.83	26.83	28.17	2.00
Av.	27.25	31.00	28.16	29.42	6.12
			Feed/gain (lb.)		
1	2.04	2.03	2.04	1.81	2.83
2	1.99	1.95	2.10	2.19	4.33
Av.	2.02	1.99	2.07	2.00	3.24
			Average daily feed consumption (lb.)		
1	2.10	2.40	2.15	1.98	0.71
2	1.82	2.01	2.01	2.20	1.08
Av.	1.96	2.20	2.08	2.09	0.90

Table 9. Experiment 1179 - Analysis of variance for gain, feed per pound gain and average daily feed intake

Source	d.f.	Mean squares		
		Gain	Feed/gain	Daily feed
Replication	1	43.7647[a]	0.3276	0.0048
Treatment	4	212.5167[b]	0.9769	0.5803[a]
Linear	1	384.2138[b]	1.9719[a]	1.0080[a]
Quadratic	1	357.1428[b]	1.3376	1.0764[a]
Cubic	1	64.5482[b]	0.4774	0.1394
Quartic	1	44.1620[a]	0.1206	0.0972
Error	4	2.8416	0.2190	0.0532
Total	9	100.5776	0.5679	0.2821

[a]Significant at P = .05 or less.

[b]Significant at P = .01 or less.

Table 10. Experiment 1179 - Summary of serum calcium, serum phosphorus, and serum alkaline phosphatase

Added vitamin A (IU/lb.)	0	500	5,000	50,000	500,000
Replication		Serum calcium (mg/100 ml)			
1	9.32	10.34	7.94	4.65	12.42
	10.34	11.53	10.04	4.16	15.38
	11.59	11.28	10.78	5.76	11.15
2	7.69	11.10	10.66	6.82	13.20
	6.01	11.34	13.08	9.80	9.36
	6.26	12.40	9.98	10.30	11.28[a]
Av.	8.54	11.33	10.41	6.92	12.13
	Serum inorganic phosphorus (mg/100 ml)				
1	9.26	9.04	8.14	11.94	8.38
	7.08	7.52	10.24	11.18	9.17
	8.89	8.34	10.12	11.36	7.16
2	9.84	9.90	10.18	11.24	9.32
	9.05	9.67	11.88	10.12	7.63
	9.98	10.33	11.56	12.08	8.80[a]
Av.	9.02	9.13	10.35	11.32	8.41
	Serum alkaline phosphatase (millimoles of nitrophenol liberated per hour)				
1	0.518	0.816	0.959	0.986	0.731
	0.618	0.803	0.717	0.862	0.568
	0.681	0.654	1.384	1.284	1.524
2	0.878	1.096	0.983	1.072	0.990
	0.700	0.552	0.796	0.787	0.404
	0.826	1.010	0.726	1.006	0.679[a]
Av.	0.704	0.822	0.912	0.970	0.819

[a]Estimated value.

Table 11. Experiment 1179 - Summary of bone ash calcium, phosphorus, and bone alkaline phosphatase

Added vitamin A (IU/lb.)	0	500	5,000	50,000	500,000
Replication		Bone calcium (% of ash)			
1	42.96	36.38	36.95	36.88	36.76
	37.58	35.96	37.84	36.70	66.91
	36.67	36.26	35.57	38.48	34.47
2	44.75	35.12	37.57	36.34	38.45
	36.86	35.18	35.83	48.59	37.12[a]
	36.70	37.38	35.78	36.58	37.78[a]
Av.	39.25	36.05	36.59	38.93	41.92
		Bone phosphorus (% of ash)			
1	10.27	8.37	12.83	12.78	11.12
	12.79	10.67	12.56	15.22	22.63
	12.37	12.30	11.61	12.36	9.68
2	14.50	11.26	13.30	13.04	14.80
	12.84	9.16	13.04	16.07	12.84[a]
	12.29	12.41	12.89	11.36	13.82[a]
Av.	12.51	10.70	12.70	13.47	14.15
		Bone alkaline phosphatase (micromoles of nitrophenol liberated per hour)			
1	34.60	22.19	31.49	41.52	23.23
	34.02	32.98	37.01	40.96	15.53
	29.92	36.34	30.64	45.22	26.24
2	31.44	31.64	24.76	28.68	12.80
	16.70	25.08	32.74	31.48	16.46
	33.33	30.46	33.29	37.57	14.63
Av.	30.00	29.78	31.66	37.57	14.63

[a]Estimated value.

Table 12. Experiment 1179 - Analysis of variance for serum calcium and inorganic phosphorus and bone calcium and phosphorus

Source	d.f.	Mean squares			
		Serum calcium	Serum phosphorus	Bone calcium	Bone phosphorus
Outcome group	5	1.1441	1.6992	28.1591	8.5093
Replication	1	0.2254	6.3113	8.8999	1.2120
OCG/Rep.	4	1.3738	0.5462	32.9740	10.3336[a]
Treatment	4	27.0914	8.2338[b]	33.1039	10.1122[b]
Linear	1	4.6259	0.5684	40.3932	21.9494[a]
Quadratic	1	2.1890	17.0460[b]	86.1941	6.0107
Cubic	1	92.7029	14.8802[b]	5.7722	9.1494[b]
Quartic	1	8.8479	0.4404	0.0560	3.3393
OCG x Treatment	19	4.7745	0.9172	42.4805	5.3344
Rep. x Treatment	4	13.8418	1.1983	27.2457	0.8937
OCG/Rep. x Treatment	15	2.3565	0.8423	46.5431	6.5186
Total	28	7.3144	2.1021	38.5836	6.5839

[a]Significant at P = .01 or less.

[b]Significant at P = .05 or less.

Table 13. Experiment 1179 - Analysis of variance for serum alkaline phosphatase and bone alkaline phosphatase

Source	d.f.	Mean squares: Serum alkaline phosphatase	Mean squares: Bone alkaline phosphatase
Outcome group	5	0.145464	63.7881
Replication	1	0.015053	217.7829[a]
OCG/Rep.	1	0.178067	25.2894
Treatment	4	0.074198	298.4457[b]
Linear	1	0.100205	152.0042[a]
Quadratic	1	0.154972	506.0737[b]
Cubic	1	0.034512	451.5527[b]
Quartic	1	0.007101	84.1524
OCG x Treatment	19	0.043071	24.7464
Rep. x Treatment	4	0.059400	17.3747
OCG/Rep. x Treatment	15	0.038716	26.7123
Total	28	0.065802	70.8181

[a]Significant at P = .05 or less.

[b]Significant at P = .01 or less.

Table 14. Experiment 1179 - Summary of bone dry matter, ether extract, and ash

Added vitamin A (IU/lb.)	0	500	5,000	50,000	500,000
Replication		Bone percent dry matter			
1	48.58	52.11	52.44	52.29	57.66
	51.75	53.12	49.40	52.40	54.22
	50.74	47.62	50.18	51.27	44.98
2	49.63	49.57	46.55	51.21	51.44
	51.84	50.36	49.60	55.18	56.62
	49.75	51.78	50.69	51.35	54.03[a]
Av.	50.38	50.76	49.81	52.28	53.16
		Bone ether extract (% of dry bone)			
1	6.97	9.94	5.39	8.42	10.76
	9.24	6.46	1.94	2.47	6.32
	7.57	5.41	10.77	8.08	1.76
2	9.57	9.02	7.92	6.02	8.79
	12.16	1.80	5.60	8.28	6.14
	6.86	11.14	3.69	6.65	7.46[a]
Av.	8.73	7.30	5.88	6.65	6.87
		Bone ash (% of dried bone)			
1	46.5	48.1	45.7	48.4	48.2
	48.8	45.4	45.1	44.6	25.2
	50.1	41.4	49.4	47.0	41.6
2	46.3	45.0	46.7	47.6	48.1
	48.6	44.8	44.7	37.3	46.3
	48.0	47.7	44.9	48.0	47.3[a]
Av.	48.0	45.4	46.1	45.5	42.8

[a]Estimated value.

Table 15. Experiment 1179 - Analysis of variance for bone dry matter, ether extract and ash

Source	d.f.	Mean squares		
		Dry matter	Ether extract	Ash
Outcome group	5	12.6742	6.3168	22.86
Replication	1	0.0236	3.0720	8.21
OCG/Rep.	4	15.8368	7.1280	26.52
Treatment	4	11.6597	6.6247	21.46
Linear	1	30.0475[a]	11.3796[a]	65.94
Quadratic	1	8.3601	12.8780[a]	0.86
Cubic	1	0.0437	0.1972[a]	17.82
Quartic	1	8.1873	2.0440	1.23
OCG x Treatment	19	5.2068	8.8851	22.66
Rep. x Treatment	4	2.4389	0.9184	30.71
OCG/Rep. x Treatment	15	5.9450	11.0096	20.52
Total	28	7.4621	8.1036	22.53

[a]Significant at P = .05 or less.

Table 16. Experiment 6327 - Summary of weight gain and feed per pound gain

Added to diet:					
Vitamin A (IU/lb.)	0	100,000	100,000	100,000	100,000
Vitamin D (IU/lb.)	0	0	50	500	5,000
Replication			Total gain (lb.)		
1	29.3	31.2	23.3	26.0[a]	21.6
	32.0	37.6	28.8	35.5	35.5
	29.4	32.0	27.9	29.4	31.8
	35.3	38.0	32.9	23.4	35.6
2	31.1	21.0	23.5[a]	16.4	27.0
	24.3	25.7	31.7[a]	32.9	29.1
	27.0	25.6	38.1	28.9	27.0
	24.0	25.6	23.0	19.6	21.1
Av.	29.0	29.6	28.6	27.8	28.6
			Feed/gain		
1	1.88	1.96	1.98	1.87	1.82
2	2.03	1.89	1.85	2.10	2.07
Av.	1.96	1.92	1.92	1.99	1.94

[a]Calculated value.

Table 17. Experiment 6327 - Analysis of variance for total gain and feed per pound gain

Source	d.f.	Mean squares	
		Total gain	Feed/gain
Outcome group	7	91.1520[a]	
Replication	1	268.3240[a]	0.0185
OCG/Rep.	6	68.6233[a]	
Treatment	4	3.6241	0.0015
Levels of Vitamin A	1	1.0890	
Levels of Vitamin D	3	4.4692	
Linear	1	6.4000	
Quadratic	1	5.9512	
Cubic	1	1.0562	
Outcome group x Treatment	26	16.7432	
Rep. x Treatment	4	32.0059	
OCG/Rep. x Treatment	22	13.9682	
Totals	37	30.5374	0.0095

[a]Significant at P = .01 or less.

Table 18. Experiment 6327 - Summary of serum calcium and inorganic phosphorus

Added to diet:					
Vitamin A (IU/lb.)	0	100,000	100,000	100,000	100,000
Vitamin D (IU/lb.)	0	0	50	500	5,000
Replication	Serum calcium (mg/100 ml)				
1	8.05	8.35	8.90	10.05[a]	8.62
	8.30	7.68	8.38	9.30	10.10
	8.45	8.05	8.82	11.02	11.28
	8.25	9.18	8.75	10.88	8.75
2	9.58	8.32	11.15	10.10	8.75
	7.48	8.95	9.76[a]	10.00	9.22
	8.15	8.25	9.02	9.30	8.95
	8.40	8.55	9.02	9.68	8.58
Av.	8.33	8.42	9.22	10.04	9.28
	Inorganic phosphorus (mg/100 ml)				
1	9.25	14.28	13.37	13.70[a]	12.58
	13.41	15.28	15.66	17.04	14.70
	14.45	13.41	14.37	15.45	14.70
	12.08	13.24	16.12	14.37	14.04
2	14.41	14.50	13.24	11.91	14.08
	13.24	14.20	14.33[a]	14.28	14.41
	14.66	14.41	14.99	12.70	12.83
	14.04	14.41	14.08	14.28	13.41
Av.	13.19	14.22	14.52	14.22	13.84

[a]Calculated value.

Table 19. Experiment 6327 - Summary of serum alkaline phosphatase and acid phosphatase

Added to diet:					
Vitamin A (IU/lb.)	0	100,000	100,000	100,000	100,000
Vitamin D (IU/lb.)	0	0	50	500	5,000
Replication	Alkaline phosphatase (millimoles of nitrophenol liberated per hour)				
1	1.350	1.470	1.461	1.544	1.402
	1.966	1.466	1.452	1.972	1.337
	1.673	1.416	1.369	1.221	1.458
	1.710	1.332	1.259	1.586	1.196
2	2.250	1.662	1.374	1.124	1.248
	1.617	1.271	1.201	1.102	1.546
	1.424	1.311	1.342	1.290	1.608
	1.748	1.546	1.332	1.524	1.650
Av.	1.717	1.434	1.349	1.420	1.431
	Acid phosphatase (millimoles of nitrophenol liberated per hour)				
1	0.2008	0.2480	0.2402	0.2506	0.2646
	0.2767	0.5147	0.6366	0.4048	0.2515
	0.3661	0.1863	0.2863	0.3022	0.1759
	0.1568	0.3491	0.2550	0.2650	0.2863
2	0.5674	0.2014	0.2053	0.3022	0.2342
	0.2945	0.1780	0.1576	0.1842	0.2452
	0.3076	0.1842	0.1524	0.1443	0.2231
	0.2902	0.2150	0.2100	0.2090	0.2008
Av.	0.3075	0.2596	0.2679	0.2578	0.2352

Table 20. Experiment 6327 - Analysis of variance for serum calcium, inorganic phosphorus, alkaline phosphatase, and acid phosphatase

Source	d.f.	Mean squares			
		Calcium	Inorganic phosphorus	Alkaline phosphatase	Acid phosphatase
Outcome group	7	0.5816	2.6621	0.047319	0.02384022
Replication	1	0.0001	0.2387	0.005523	0.03665697
OCG/Rep.	6	0.6786	3.0660	0.054285	0.02170413
Treatment	4	3.9654[a]	2.0808	0.162250[b]	0.00556681
Levels of Vitamin A	1	5.2316[a]	6.4843[b]	0.610090[a]	0.01756448
Levels of Vitamin D	3	3.5266[a]	0.6129	0.012969	0.00156759
Linear	1	4.6546[a]	0.8080	0.001476	0.00277600
Quadratic	1	4.9220[a]	0.9146	0.018334	0.00191270
Cubic	1	1.0033	0.1161	0.019100	0.00001452
Outcome group x Treatment	26	0.5542	1.4010	0.046825	0.01003617
Rep. x Treatment	4	1.0328	3.6138	0.070258	0.02561844
OCG/Rep. x Treatment	22	0.4671	0.9986	0.042564	0.00720303
Totals	37	0.9281	1.7130	0.059397	0.01216457

[a]Significant at P = .01 or less.

[b]Significant at P = .05 or less.

Table 21. Experiment 6327 - Summary of bone alkaline phosphatase and acid phosphatase

Added to diet:					
Vitamin A (IU/lb.)	0	100,000	100,000	100,000	100,000
Vitamin D (IU/lb.)	0	0	50	500	5,000
Replication		Alkaline phosphatase (micromoles of nitrophenol liberated per hour)			
1	34.38	17.72	21.02	31.10	23.84
	39.23	20.89	28.03	23.24	21.59
	26.40	22.12	27.10	14.63	21.60
	34.26	25.64	28.32	37.25	21.54
2	24.22	28.62	17.40	27.50	23.21
	27.05	24.60	16.07	22.36	29.35
	15.82	28.58	26.23	36.46	24.65
	37.46	26.96	17.60	30.72	23.38
Av.	29.85	24.39	22.72	27.91	23.64
		Bone acid phosphatase (micromoles of nitrophenol liberated per hour)			
1	0.774	0.696	0.303	0.839	0.599
	0.710	0.542	0.252	0.558	1.162
	0.782	2.796	0.542	0.626	0.710
	0.489	1.730	0.462	0.408	1.643
2	0.448	1.926	1.558	4.380	2.798
	0.654	2.036	0.569	1.170	1.162
	0.489	1.730	0.598	0.739	1.737
	2.440	0.986	0.928	1.201	0.558
Av.	0.848	1.555	0.652	1.240	1.296

Table 22. Experiment 6327 - Summary of bone ether extract and bone ash

Added to diet:					
Vitamin A (IU/lb.)	0	100,000	100,000	100,000	100,000
Vitamin D (IU/lb.)	0	0	50	500	5,000
Replication	Bone ether extract (% of dry bone)				
1	7.53	7.80	6.06	3.00	9.31
	10.02	4.05	3.37	1.24	2.77
	6.96	3.10	7.44	5.63	8.52
	4.14	10.64	8.89	6.07	8.89
2	7.88	4.23	5.04	10.27	5.11
	7.96	4.20	10.84	10.48	3.54
	8.75	2.16	9.37	10.37	8.91
	3.12	13.76	13.00	11.29	1.87
Av.	7.04	6.24	8.00	7.29	6.12
	Bone ash (% of dry bone)				
1	38.62	39.99	41.41	41.55	35.65
	41.99	44.26	41.03	39.50	53.82
	41.39	41.41	41.96	45.97	30.83
	44.31	46.82	46.88	41.96	53.68
2	37.05	36.92	43.58	38.74	40.31
	39.72	39.70	38.26	42.90	42.31
	38.72	42.00	42.34	42.45	43.88
	39.53	37.16	43.63	46.66	39.55
Av.	40.17	41.03	42.39	42.47	42.50

Table 23. Experiment 6327 - Analysis of variance for bone alkaline phosphatase, acid phosphatase, ether extract, and ash

Source	d.f.		Mean squares: Alkaline phosphatase	Acid phosphatase	Ether extract	Ash
Outcome group	7		24.2267	1.2241	8.7022	32.3447
Replication		1	3.3990	3.2970[a]	17.8490	35.3816
OCG/Rep.		6	27.6980	0.8786	7.1777	31.8386
Treatment	4		73.8501	1.0564	4.8591	9.0028
Levels of Vitamin A		1	172.1420[a]	0.7290	0.1113	23.8625
Levels of Vitamin D		3	41.0862	1.1656	6.4417	4.0497
Linear		1	3.4751	0.0142	0.4752	8.0775
Quadratic		1	13.4421	1.8422	17.2578	3.4650
Cubic		1	106.3412	1.6402	1.5920	0.6064
Outcome group x Treatment	26		36.3228	0.5549	12.0168	15.6723
Rep. x Treatment		4	73.6612	0.3328	25.5467	6.2925
OCG/Rep. x Treatment		22	30.0998	0.5919	9.7618	17.2356
Totals	37		38.0007	0.7265	10.6877	17.9808

[a]Significant at P = .05 or less.

Table 24. Experiment 6327 - Summary of metatarsal epiphysis calcium and diaphysis calcium

Added to diet:					
Vitamin A (IU/lb.)	0	100,000	100,000	100,000	100,000
Vitamin D (IU/lb.)	0	0	50	500	5,000
Replication		Epiphysis calcium (% of ash)			
1	19.86	53.61	27.13	21.17	28.85
	35.24	34.28	39.21	52.64	35.71
	52.81	32.07	31.54	35.54	31.35
	33.82	31.37	33.29	24.69	38.63
2	31.81	28.12	31.90	25.32	30.02
	28.41	29.18	17.89	34.36	32.92
	32.58	34.22	40.37	32.62	29.58
	32.03	33.39	31.64	32.75	33.98
Av.	33.32	34.53	31.62	32.39	32.63
		Diaphysis calcium (% of ash)			
1	23.38	44.66	46.97	23.43	30.94
	34.08	37.82	30.69	42.92	34.85
	36.69	35.33	35.25	34.51	34.25
	35.21	36.98	32.89	24.80	32.98
2	35.20	28.45	33.73	30.89	26.21
	34.52	35.34	37.12	34.15	35.08
	29.48	30.08	30.63	34.25	29.40
	29.65	26.38	24.82	38.82	33.79
Av.	32.28	34.38	34.01	31.72	32.19

Table 25. Experiment 6327 - Summary of metatarsal epiphysis phosphorus and diaphysis phosphorus

Added to diet:					
Vitamin A (IU/lb.)	0	100,000	100,000	100,000	100,000
Vitamin D (IU/lb.)	0	0	50	500	5,000
Replication	Epiphysis phosphorus (% of ash)				
1	15.04	22.81	14.78	9.59	14.83
	15.11	15.08	16.81	20.77	14.64
	17.26	14.84	14.98	14.99	15.32
	15.00	15.17	15.16	10.99	14.76
2	14.98	15.35	14.81	14.18	14.89
	14.91	15.41	14.51	14.79	14.84
	13.76	15.04	14.96	14.62	14.19
	14.32	15.05	14.82	15.74	14.38
Av.	15.05	16.09	15.10	14.46	14.74
	Diaphysis phosphorus (% of ash)				
1	17.64	21.52	17.92	11.80	17.08
	16.92	17.93	16.12	21.46	17.32
	16.54	16.78	17.57	16.46	17.21
	17.65	16.48	16.48	12.75	16.74
2	16.34	17.49	17.01	16.97	16.65
	17.13	17.26	17.63	18.42	17.18
	16.95	16.90	17.00	16.55	15.90
	17.00	16.76	16.50	17.78	17.14
Av.	17.02	17.64	17.03	16.52	16.90

Table 26. Experiment 6327 - Analysis of variance for epiphysis and diaphysis calcium and phosphorus

Source	d.f.	Mean squares			
		Epiphysis calcium	Diaphysis calcium	Epiphysis phosphorus	Diaphysis phosphorus
Outcome group	7	68.9255	34.4222	2.4658	1.6474
Replication	1	121.5220	91.9303	3.8938	0.0009
OCG/Rep.	6	60.1594	24.8376	2.2278	1.9218
Treatment	4	9.6106	11.4260	3.0558	1.2890
Levels of Vitamin A	1	1.7851	4.0864	0.0176	0.0000
Levels of Vitamin D	3	12.2191	13.8726	4.0685	1.7187
Linear	1	9.7417	31.4619	8.8172	2.9539
Quadratic	1	19.8765	1.3903	3.2512	1.9602
Cubic	1	7.0392	8.7656	0.1369	0.2418
Outcome group x Treatment	26	58.8109	27.4353	4.4200	2.8345
Rep. x Treatment	4	7.6472	28.6567	5.7775	2.3440
OCG/Rep. x Treatment	22	67.3382	27.2317	4.1938	2.9163
Totals	37	55.5802	27.0474	3.9293	2.4629

Table 27. Experiment 6311 - Summary of weight gain and feed per pound gain

Added vitamins:						
Vitamin A (IU/lb.)	0	0	0	100,000	100,000	100,000
Vitamin D (IU/lb.)	500	5,000	50,000	500	5,000	50,000
Replication			Total gain (lb.)			
1	20.7	27.2	32.9	27.4	24.1	22.7[a]
	31.9	30.9	26.7	28.0	29.5	27.7
	34.8	26.3	32.9	23.8	28.1	27.0
	30.8	33.8	29.6	26.3	24.6	23.7
2	32.0	38.5	34.0	37.5	28.5	33.5
	42.0	42.0	33.5	37.5	45.0	35.5
	44.0	43.5	38.0	32.0	34.0	28.0
	40.0	34.0	35.5	33.0	26.5	33.5
Av.	34.5	34.5	34.3	30.7	30.0	29.0
			Feed/gain			
1	1.80	1.94	2.00	1.92	1.82	1.94
2	2.09	1.89	2.06	2.02	1.86	2.03
Av.	1.94	1.92	2.03	1.97	1.84	1.98

[a]Calculated value.

Table 28. Experiment 6311 - Analysis of variance for gain and feed per pound of gain

Source	d.f.	Mean squares	
		Gain	Feed/gain
Outcome group	7	121.5271[a]	
Replication	1	675.7502[a]	0.0234
OCG/Rep.	6	29.1566	
Treatment	5	51.3164[b]	0.0085
Vitamin A vs none	1	243.4502[a]	0.0030
Vitamin D levels	2	4.7425	0.0172
Linear	1	9.0312	0.0050
Quadratic	1	0.4538	0.0294
Vitamin A X D	2	1.8234	0.0026
OCG x Treatment	34	17.6855	
Rep. x Treatment	5	16.5797	0.0315
OCG/Rep. x Treatment	29	17.8761	
Total	46	37.1430	0.0976

[a]Significant at P = .01 or less.

[b]Significant at P = .05 or less.

Table 29. Experiment 6311 - Summary of serum calcium and inorganic phosphorus

Added vitamins:						
Vitamin A (IU/lb.)	0	0	0	100,000	100,000	100,000
Vitamin D (IU/lb.)	500	5,000	50,000	500	5,000	50,000
Replication	Serum calcium (mg/100 ml)					
1	5.2	8.8	6.1	6.2	7.1	9.5[a]
	5.6	9.4	6.2	5.7	5.8	9.0
	5.7	9.8	7.0	6.0	5.2	9.5
	6.8	3.8	8.4	5.6	4.8	9.0
2	13.1	12.0	10.6	12.8	16.2	10.7
	12.0	13.8	11.5	13.2	11.7	9.2
	10.0	14.8	21.5	11.5	12.0	9.3
	10.3	11.3	29.0	14.0	12.4	10.2
Av.	8.6	10.5	12.5	9.4	9.4	9.5
	Serum phosphorus (mg/100 ml)					
1	14.33	15.33	16.29	16.66	13.83	15.48[a]
	12.42	14.41	14.83	15.08	13.74	15.25
	13.66	13.66	13.92	16.00	13.50	14.55
	14.66	16.33	16.00	15.24	14.74	14.42
2	17.12	19.41	17.08	17.30	17.42	18.00
	18.16	17.46	17.92	15.87	17.42	16.00
	17.16	16.30	16.58	17.33	15.88	16.33
	18.00	15.34	15.20	17.50	14.84	15.42
Av.	15.68	16.03	15.98	16.37	15.17	15.78

[a]Calculated value.

Table 30. Experiment 6311 - Summary of serum alkaline phosphatase and serum acid phosphatase

Added vitamins:						
Vitamin A (IU/lb.)	0	0	0	100,000	100,000	100,000
Vitamin D (IU/lb.)	500	5,000	50,000	500	5,000	50,000
Replication	Serum alkaline phosphatase (millimoles of nitrophenol liberated per hour)					
1	1.3757	1.6984	1.2252	1.4422	1.1518	1.5971[a]
	0.9191	2.2184	1.5934	1.8938	1.6424	1.9052
	1.0230	1.6233	1.1519	1.3206	1.7065	1.6830
	1.1850	1.1653	1.0399	0.8701	1.0374	1.1452
2	2.1177	2.3796	2.0434	2.2718	2.1098	2.2664
	2.1678	2.0981	2.0460	2.2516	1.7696	1.9920
	2.1284	2.0868	2.3559	2.4392	2.4018	2.3024
	2.2756	1.9880	2.0194	2.6522	2.1542	1.9018
Av.	1.6490	1.9072	1.6844	1.8927	1.7467	1.8491
	Serum acid phosphatase (millimoles of nitrophenol liberated per hour)					
1	0.1450	0.0963	0.1631	0.5052	0.1406	0.2061[a]
	0.1818	0.1790	0.1978	0.1686	0.1748	0.2520
	0.1029	0.1418	0.1482	0.2513	0.3016	0.1319
	0.0942	0.1153	0.1734	0.1224	0.2248	0.1200
2	0.1654	0.1444	0.2626	0.2638	0.1990	0.2224
	0.1702	0.2287	0.2993	0.1946	0.1158	0.1786
	0.1872	0.1668	0.1932	0.1476	0.1072	0.2192
	0.2925	0.2287	0.3087	0.2714	0.1548	0.2340
Av.	0.1674	0.1626	0.2183	0.2406	0.1773	0.1955

[a]Calculated value.

Table 31. Experiment 6311 - Analysis of variance for serum alkaline phosphatase, acid phosphatase, calcium and inorganic phosphorus

Source	d.f.	Mean squares			
		Alkaline phosphatase	Acid phosphatase	Calcium	Inorganic phosphorus
Outcome group	7	1.220282[a]	0.005966	67.8050[a]	10.2007[a]
Replication	1	7.211841[a]	0.007957	449.5752[a]	55.2982[a]
OCG/Rep.	6	0.221688[a]	0.005635	4.1766	2.6845[b]
Treatment	5	0.097068	0.007574	15.3597	1.2995
Vitamin A vs none	1	0.081939	0.005659	14.1919	0.1850
Vitamin D levels	2	0.018101	0.006748	17.0508	0.7608
Linear	1	0.000134	0.000067	34.0312	0.1845
Quadratic	1	0.036068	0.013428	0.0704	1.3372
Vitamin A x D	2	0.183599[a]	0.009359	14.2525	2.3954
OCG x Treatment	34	0.056125	0.005148	11.4678	0.8994
Rep. x Treatment	5	0.120761	0.008560	24.1487	1.7803
OCG/Rep. x Treatment	29	0.044980	0.004560	9.2815	0.7475
Total	46	0.237729	0.005536	20.4639	2.3583

[a]Significant at P = .01 or less.

[b]Significant at P = .05 or less.

Table 32. Experiment 6318 - Summary of weight gain and feed per pound gain

Added to diet:				
Vitamin A (IU/lb.)	100,000	100,000	100,000	100,000
Vitamin C (IU/lb.)	0	100	0	100
Thyroprotein (mg/lb.)	0	0	100	100
Replication		Total gain (lb.)		
1	44.0	44.5	28.5	33.0
	25.0	30.5	27.0	25.5
	36.5	30.0	32.5	27.5
	20.5	28.0	24.0	24.0
2	33.5	31.5	44.5	34.5
	32.0	19.0	28.5[a]	24.3[a]
	34.5	41.0	32.0	33.0
	25.5	27.0	29.0	25.5
3	33.5	29.5	30.5	33.0[a]
	35.5	34.0	32.0	33.2[a]
	44.0	24.5	25.5	32.5
	35.5	28.5	34.5	28.0
Av.	33.3	30.7	30.7	29.5
		Feed/gain		
1	2.02	2.00	2.21	2.37
2	1.87	2.18	2.28	2.18
3	1.88	2.02	2.12	2.29
Av.	1.92	2.07	2.20	2.28

[a]Calculated value.

Table 33. Experiment 6318 - Analysis of variance for gain and feed per pound gain

Source	d.f.	Mean squares	
		Gain	Feed/gain
Outcome group	11	72.6479[a]	
Replication	2	17.3327	0.0055
OCG/Rep.	9	84.9402[a]	
Treatment	3	31.5191	0.0741[a]
Vit. C (0 vs 100 mg/lb.)	1	45.0469	0.0363
Thyroprotein (0 vs 100 mg/lb.)	1	43.1302	0.1826[a]
Vit. C x Thyroprotein	1	6.3802	0.0033
OCG x Treatment	30	24.9138	
Rep. x Treatment	6	31.2924	0.0089
OCG/Rep. x Treatment	24	23.3192	
Total	44	1641.0998	0.0261

[a]Significant at P = .05 or less.

Table 34. Experiment 6318 - Summary of serum calcium and inorganic phosphorus

Added to diet:								
Vitamin A (IU/lb.)	100,000	100,000	100,000	100,000	100,000	100,000	100,000	100,000
Vitamin C (mg/lb.)	0	100	0	100	0	100	0	100
Thyroprotein (mg/lb.)	0	0	100	100	0	0	100	100
Replication	Serum calcium (mg/100 ml)				Serum inorganic phosphorus (mg/100 ml)			
1	10.9	10.8	10.1	10.1	14.04	16.00	14.66	14.25
	10.9	10.4	11.2	19.0	13.21	12.71	15.54	16.16
	12.6	9.8	9.6	11.0	15.50	14.88	15.21	15.75
	13.4	9.8	11.2	9.8	13.66	14.25	14.83	15.50
2	8.2	7.4	9.0[a]	8.6	8.25	9.33	8.50[a]	9.50[a]
	9.0	8.0	9.1[a]	9.6[a]	14.66	8.91	11.32[a]	12.27[a]
	8.8	9.6	10.0[a]	10.9	11.50	13.74	11.82[a]	12.08
	8.1	8.1	7.9	9.0	9.08	9.66	9.08	10.66
3	8.9	9.0	8.0	8.0	10.75	12.24	15.91	10.66
	7.9	7.4	8.0	7.6[a]	10.50	12.99	14.91	11.37[a]
	8.5	8.6	7.0	7.9[a]	8.50	11.74	10.33	8.76[a]
	8.2	8.3	8.1	8.5	11.66	12.99	12.50	11.83
Av.	9.6	8.9	9.1	10.0	11.78	12.45	12.89	12.40

[a]Calculated value.

Table 35. Experiment 6318 - Summary of serum alkaline phosphatase and serum acid phosphatase

Added to diet:								
Vitamin A (IU/lb.)	100,000	100,000	100,000	100,000	100,000	100,000	100,000	100,000
Vitamin C (mg/lb.)	0	100	0	100	0	100	0	100
Thyroprotein (mg/lb.)	0	0	100	100	0	0	100	100
Replication	Alkaline phosphatase (millimoles of nitrophenol liberated per hour)				Acid phosphatase (millimoles of nitrophenol liberated per hour)			
1	2.070	2.216	2.385	1.991	0.112	0.149	0.157	0.127
	2.059	1.968	2.604	2.048	0.151	0.115	0.146	0.116
	1.962	1.608	2.535	1.946	0.141	0.123	0.125	0.119
	1.834	1.950	2.179	2.095	0.222	0.176	0.204	0.190
2	2.085	1.614	2.574	2.710	0.280	0.272	0.374	0.478
	2.916	2.328	3.692[a]	3.703[a]	0.308	0.444	0.393[a]	0.601[a]
	2.203	2.328	3.348[a]	3.382	0.468	0.394	0.454[a]	0.675
	2.116	1.954	3.439	3.300	0.302	0.479	0.320	0.619
3	2.072	2.185	2.400	2.662	0.387	0.289	0.244	0.294
	2.158	2.332	3.050	2.905[a]	0.282	0.276	0.308	0.294[a]
	1.638	3.006	1.585	2.468[a]	0.372	0.400	0.302	0.363[a]
	1.850	1.886	2.142	2.300	0.355	0.233	0.276	0.311
Av.	2.080	2.115	2.661	2.626	0.282	0.279	0.275	0.349

[a]Calculated value.

Table 36. Experiment 6318 - Analysis of variance for serum calcium, inorganic phosphorus, alkaline phosphatase, and acid phosphatase

Source	d.f.	Mean squares			
		Calcium	Inorganic phosphorus	Alkaline phosphatase	Acid phosphatase
Outcome group	11	10.0157[a]	18.1214[a]	0.5410[a]	0.0640[a]
Replication	2	44.2182[a]	72.8051[a]	1.1445[a]	0.3176[a]
OCG/Replication	9	2.4151	5.9695[b]	0.2797	0.0077
Treatment	3	2.8564	2.5133	1.1975[a]	0.0149
Vit. C (0 vs 100 mg/lb.)	1	0.1408	0.1074	0.0000	0.0152
Thyroprotein (0 vs 100 mg/lb.)	1	0.9075	3.3550	3.5779[a]	0.0120
Vit. C x Thyroprotein	1	7.5209	4.0774	0.0145	0.0174
OCG x Treatment	28	2.3332	2.3518	0.1672	0.0060
Rep. x Treatment	6	1.7495	4.1200	0.4464	0.0196
OCG/Rep. x Treatment	22	2.4924	1.8696	0.0911	0.0023
Total	42	4.3827	6.4935	0.3387	0.0218

[a]Significant at P = .01 or less.

[b]Significant at P = .05 or less.

Table 37. Experiment 6421 - Summary of weight gain and feed per pound gain

Added to diet:								
Vit. A (IU/lb.)	150,000	150,000	150,000	150,000	150,000	150,000	150,000	150,000
Vit. D (IU/lb.)	500	500	500	500	50,000	50,000	50,000	50,000
Vit. K (mg/lb.)	0	15	0	15	0	15	0	15
Vit. E (IU/lb.)	0	0	100	100	0	0	100	100
Replication				Total gain (lb.)				
1	16.3	12.0	3.1	10.9	19.5	11.0	12.1	13.1
	17.1	21.6	17.0	17.8	16.2	17.2	20.1	20.5
	12.8	13.8	16.2	16.5	19.8	11.1	13.6	17.0
	14.3	10.5	11.3	16.9	11.7	10.1	16.5	17.7
2	17.4	14.3	18.2	21.9	19.1	16.3	20.1	13.4
	21.5	20.0	18.0	16.5	14.0	17.5	19.5	16.5
	21.0	20.5	16.0	7.9	12.4	23.0	15.7	20.0
	17.2	15.0	16.7	20.0	17.5	14.6	17.5	18.0
Av.	17.2	16.0	14.6	16.0	16.3	15.1	16.9	17.0
				Feed/gain				
1	2.11	2.15	2.26	2.06	2.01	2.76	1.99	2.02
2	1.89	1.78	1.88	2.04	2.03	1.87	1.81	1.91
Av.	2.00	1.96	2.07	2.05	2.02	2.32	1.90	1.96

Table 38. Experiment 6421 - Analysis of variance for total gain and feed per pound gain

Source	d.f.	Mean squares	
		Gain	Feed/gain
Outcome group	7	39.8232[a]	
Replication	1	104.8064[a]	0.2889[b]
OCG/Replication	6	28.9926[b]	
Treatment	7	6.9636	0.0312
Levels of Vit. D	1	2.2877	0.0033
Levels of Vit. E	1	0.0002	0.0248
Levels of Vit. K	1	0.6202	0.0233
Vit. D x Vit. E	1	25.8826	0.0977
Vit. K x Vit. D	1	1.6578	0.0430
Vit. E x Vit. K	1	16.3013	0.0115
Vit. D x Vit. E x Vit. K	1	1.9953	0.0150
Outcome group x Treatment	49	11.3158	
Rep. x Treatment	7	11.8232	0.0421
OCG/Rep. x Treatment	42	11.2312	
Total	63(15)[c]	13.9997	0.0535

[a]Significant at P = .01 or less.

[b]Significant at P = .05 or less.

[c]Total d.f. for feed/gain.

Table 39. Experiment 6421 - Summary of serum calcium and serum inorganic phosphorus

Added to diet:								
Vit. A (IU/lb.)	150,000	150,000	150,000	150,000	150,000	150,000	150,000	150,000
Vit. D (IU/lb.)	500	500	500	500	50,000	50,000	50,000	50,000
Vit. K (mg/lb.)	0	15	0	15	0	15	0	15
Vit. E (IU/lb.)	0	0	100	100	0	0	100	100
Replication				Serum calcium (mg/100 ml)				
1	9.52	9.60	9.32	10.62	9.98	7.80	9.42	11.60
	8.15	11.15	9.95	11.15	10.40	10.52	10.90	11.60
	7.00	10.95	9.22	10.22	9.22	9.75	9.52	11.48
	5.68	10.48	9.72	11.38	7.20	9.22	10.98	11.18
2	10.45	8.38	9.60	8.68	9.45	9.75	8.18	8.55
	10.32	9.85	11.18	9.60	9.70	8.78	8.20	10.05
	10.35	9.28	9.20	9.38	9.88	8.75	8.38	8.18
	10.98	8.85	10.35	8.88	10.40	8.75	9.30	10.95
Av.	9.06	9.82	9.69	9.99	9.53	9.16	9.36	10.45
				Serum inorganic phosphorus (mg/100 ml)				
1	11.28	10.18	9.20	11.56	11.42	11.39	10.69	10.35
	11.98	11.50	12.18	10.01	8.10	11.62	12.72	10.86
	10.44	10.63	10.92	11.16	11.20	10.32	8.22	11.81
	9.78	9.68	10.46	11.48	8.66	11.50	9.51	9.76
2	12.32	10.01	11.33	10.97	9.28	12.38	8.89	12.18
	12.06	10.58	11.45	10.46	10.86	11.50	9.84	10.63
	12.88	11.31	12.23	10.35	9.42	12.12	9.79	10.91
	12.57	10.69	11.42	10.18	9.34	12.18	8.63	11.33
Av.	11.66	10.57	11.15	10.77	9.78	11.63	9.79	10.98

Table 40. Experiment 6421 - Summary of serum alkaline phosphatase and serum acid phosphatase

Added to diet:								
Vit. A (IU/lb.)	150,000	150,000	150,000	150,000	150,000	150,000	150,000	150,000
Vit. D (IU/lb.)	500	500	500	500	50,000	50,000	50,000	50,000
Vit. K (mg/lb.)	0	15	0	15	0	15	0	15
Vit. E (IU/lb.)	0	0	100	100	0	0	100	100
Replication	Alkaline phosphatase (millimoles of nitrophenol liberated per hour)							
1	1.252	1.910	0.640	1.135	1.368	1.822	1.524	1.226
	1.475	1.464	1.229	1.191	1.134	1.272	1.062	1.426
	1.618	1.218	1.058	1.178	1.207	1.095	1.179	1.560
	1.640	1.535	1.409	1.200	1.284	1.317	1.009	1.051
2	1.606	1.616	1.255	1.275	1.054	1.711	1.110	1.593
	1.238	1.254	1.266	1.236	1.504	1.618	1.344	1.402
	1.172	1.432	0.984	1.353	1.090	1.456	1.241	1.238
	1.345	1.307	1.035	1.036	1.391	1.826	1.068	1.618
Av.	1.418	1.467	1.110	1.200	1.254	1.515	1.192	1.389
	Acid phosphatase (millimoles of nitrophenol liberated per hour)							
1	0.213	0.210	0.139	0.182	0.224	0.297	0.239	0.239
	0.280	0.246	0.297	0.262	0.254	0.266	0.261	0.368
	0.260	0.191	0.236	0.202	0.324	0.152	0.186	0.248
	0.236	0.256	0.242	0.230	0.220	0.212	0.246	0.356
2	0.251	0.238	0.262	0.202	0.213	0.234	0.258	0.194
	0.206	0.202	0.242	0.213	0.190	0.190	0.226	0.225
	0.227	0.178	0.184	0.212	0.190	0.210	0.217	0.210
	0.214	0.146	0.230	0.220	0.203	0.185	0.248	0.328
Av.	0.236	0.208	0.229	0.215	0.227	0.218	0.235	0.271

Table 41. Experiment 6421 - Analysis of variance for serum calcium, inorganic phosphorus, alkaline phosphatase, and acid phosphatase

Source	d.f.	Mean squares			
		Calcium	Inorganic phosphorus	Alkaline phosphatase	Acid phosphatase
Outcome group	7	1.4210	0.8802	0.023060	0.004679[a]
Replication	1	2.7639	1.4160	0.015190	0.010661[a]
OCG/Replication	6	1.1972	0.7909	0.024344	0.003682[b]
Treatment	7	1.6762	4.2197[b]	0.175094[a]	0.002937
Levels of Vit. D	1	0.0028	3.9204[b]	0.023947	0.003969
Levels of Vit. E	1	3.6960	0.9264	0.581406[a]	0.003691
Levels of Vit. K	1	3.1773	2.4492	0.357006[a]	0.000203
Vit. D x Vit. E	1	0.0945	0.1089	0.150544	0.003660
Vit. K x Vit. D	1	0.1105	20.2725[a]	0.101124	0.004624
Vit. E x Vit. K	1	0.9752	0.0042	0.000452	0.003451
Vit. D x Vit. E x Vit. K	1	3.6768	1.8564	0.011183	0.000962
Outcome group x Treatment	49	1.3354	0.9345	0.042844	0.001391
Rep. x Treatment	7	5.6790	1.4359	0.039022	0.001263
OCG/Rep. x Treatment	42	0.6115	1.4609	0.043482	0.001413
Totals	63	1.3828	1.2935	0.55338	0.001928

[a]Significant at P = .01 or less.

[b]Significant at P = .05 or less.

Table 42. Experiment 6409 - Summary of total weight gain and feed per pound of gain

Dehydrated alfalfa (%)	0	5	10	0	0
Beet pulp (%)	0	0	0	5	10
Replication			Total gain (lb.)		
1	24.75	33.50	29.50	32.00	29.62
2	32.52	32.98	30.70	31.82	32.05
3	32.32	29.92	28.17	31.95	27.12
Av.	29.86	31.80	29.46	31.92	29.60
			Feed/gain		
1	2.10	2.41	2.04	2.06	2.18
2	2.02	2.13	2.08	2.07	2.10
3	1.91	1.98	1.96	2.12	2.39
Av.	2.01	2.17	2.03	2.08	2.22

Table 43. Experiment 6409 - Analysis of variance for total gain and feed per pound gain

Source	Mean squares	
	Gain	Feed/gain
Replication	8.3203	0.0112
Treatment	4.5164	0.0257
1 vs 2+3+4+5	1.6567	0.0327
Dehydrated vs beet pulp	0.0520	0.0085
Low fiber vs high fiber	16.3567	0.0000
Other	0.0001	0.0616
Error	6.3187	0.0182
Total	6.0897	0.0193

Table 44. Experiment 6404 - Summary of weight gain, feed per pound gain, and bone ash

Added to diet:						
Vitamin A (IU/lb.)	0	0	0	400,000	100,000	3,000
Carotene (mg/lb.)	750.2	187.3	5.675	0	0	0
Replication						
	Total gain (lb.)					
1	16.0	12.5	16.5	6.0	15.5	18.0
	21.1[a]	19.5	19.0	10.5	20.0	11.5
	25.0	22.5	16.0	11.3[a]	22.0	14.5
2	21.0	15.5	16.0	5.5	17.5	22.0
	16.0	16.0	23.5	12.0	10.5	16.5
	16.5	17.0	19.5	11.2	11.5	16.8
Av.	19.3	17.1	18.4	9.4	16.1	16.6
	Feed/gain					
1	1.87	2.05	2.10	2.53	2.02	2.06
2	1.77	1.97	1.80	2.04	2.18	2.08
Av.	1.82	2.01	1.95	2.28	2.10	2.07
	Bone ash (% of dry bone)					
1	35.7	35.7	37.6	29.9	32.8	34.4
	39.3	26.0	35.8	33.0	42.2	33.8
	35.3	40.1	39.2	35.4	40.1	34.6
2	39.7	36.7	26.8	33.3	30.8	34.7
	35.1	32.8	29.0	34.6	28.8	33.3
	24.6	34.2	31.9	26.4	33.2	31.7
Av.	35.0	34.2	33.4	32.1	34.6	33.8

[a]Calculated value.

Table 45. Experiment 6404 - Summary of serum calcium and inorganic phosphorus

Added to diet:						
Vitamin A (IU/lb.)	0	0	0	400,000	100,000	3,000
Carotene (mg/lb.)	750.2	187.3	5.675	0	0	0
Replication			Serum calcium (mg/100 ml)			
1	11.62	11.28	10.22	6.00	10.82	11.15
	11.08	10.90	10.75	9.62	11.28	9.95
	10.88	11.58	10.75	4.30	12.32	11.90
2	8.25	11.08	11.38	6.62	10.90	11.12
	9.95	11.82	11.92	10.08	11.02	11.32
	10.20	13.12	12.00	10.60	11.02	11.02
Av.	10.33	11.63	11.17	7.87	11.23	11.08
			Serum inorganic phosphorus (mg/100 ml)			
1	10.04	8.94	9.62	7.74	9.54	10.85
	8.66	10.41	11.48	10.01	11.25	7.71
	11.81	11.19	9.58	6.94	11.19	10.66
2	10.12	7.76	9.22	14.54	9.02	9.36
	7.94	9.39	11.95	10.24	7.90	8.46
	9.79	9.90	11.30	11.44	7.20	10.01
Av.	9.73	9.60	10.52	10.15	9.35	9.51

Table 46. Experiment 6404 - Analysis of variance for total gain, feed per pound gain, serum calcium, and serum inorganic phosphorus

Source	d.f.	Mean squares			
		Gain	Feed/gain	Serum calcium	Inorganic phosphorus
Outcome group	5	13.6216		1.7547	0.7369
Replication	1	4.6225	0.0520	1.3689	0.1202
OCG/Replication	4	15.8714		1.8512	0.8911
Treatment	5	73.6583[a]	0.0488	11.4202[a]	1.1788
Vitamin A vs carotene	1	161.7137[a]	0.1544	8.7419[b]	0.7056
Levels	2	31.8853	0.0026	19.2161[a]	1.0331
3,000 vs 100,000 + 400,000 IU	1	31.3368	0.0051	5.9053	0.7688
100,000 vs 400,000 IU	1	32.4338	0.0000	32.5268[a]	1.2974
Interaction	2	71.4036[a]	0.0422	4.9635	1.5610
OCG x Treatment	25(23)[c]	13.5071		1.6315	3.1206
Rep. x Treatment	5	19.6992	0.0269	3.1137	7.2797
OCG/Rep. x Treatment	20(18)[c]	11.7871		1.2609	2.0808
Total	35(33)[c]	22.6383	0.0391	3.0475	2.5027

[a]Significant at P = .01 or less.

[b]Significant at P = .05 or less.

[c]Degrees of freedom for Gain.

Table 47. Experiment 6404 - Summary of serum alkaline phosphatase and acid phosphatase

Added to diet:						
Vitamin A (IU/lb.)	0	0	0	400,000	100,000	3,000
Carotene (mg/lb.)	750.2	187.3	5.675	0	0	0
Replication	Alkaline phosphatase (millimoles of nitrophenol liberated per hour)					
1	2.732	2.136	2.167	1.954	1.876	2.732
	2.541	1.837	2.543	2.336	1.980	2.984
	2.241	2.241	2.979	1.061	1.815	2.693
2	2.050	2.606	2.404	0.940	1.728	1.974
	2.506	2.295	2.336	1.720	1.956	2.122
	2.115	2.430	2.395	1.876	1.902	2.304
Av.	2.364	2.258	2.471	1.648	1.876	2.468
	Acid phosphatase (millimoles of nitrophenol liberated per hour)					
1	0.244	0.208	0.411	0.221	0.322	0.350
	0.192	0.206	0.383	0.139	0.209	0.478
	0.327	0.200	0.424	0.127	0.251	0.375
2	0.384	0.421	0.281	0.500	0.251	0.199
	0.280	0.597	0.274	0.303	0.247	0.210
	0.337	0.811	0.343	0.597	0.206	0.370
Av.	0.294	0.407	0.353	0.314	0.248	0.330

Table 48. Experiment 6404 - Summary of bone alkaline phosphatase and acid phosphatase

Added to diet:						
Vitamin A (IU/lb.)	0	0	0	400,000	100,000	3,000
Carotene (mg/lb.)	750.2	187.3	5.675	0	0	0
Replication	Alkaline phosphatase (micromoles of nitrophenol liberated per hour)					
1	5.014	5.014	2.800	2.307	4.868	4.937
	5.032	4.868	2.950	2.816	2.656	5.090
	2.406	3.050	5.090	1.183	2.127	3.967
2	2.656	4.563	2.878	2.759	5.013	4.868
	2.920	2.786	4.563	2.385	5.013	5.013
	3.715	4.868	2.680	2.558	4.750	5.090
Av.	3.634	4.192	3.493	2.335	4.071	4.828
	Acid phosphatase (micromoles of nitrophenol liberated per hour)					
1	3.704	0.576	0.597	0.599	0.688	0.651
	1.525	0.676	0.724	1.273	0.616	1.583
	1.077	0.830	3.854	0.492	0.578	1.072
2	0.484	1.160	0.724	1.116	0.894	0.858
	0.594	2.703	0.834	0.654	0.781	0.936
	0.742	1.164	1.232	0.899	0.708	1.112
Av.	1.354	1.185	1.328	0.839	0.711	1.035

Table 49. Experiment 6404 - Analysis of variance for serum alkaline phosphatase, serum acid phosphatase, bone alkaline phosphatase, bone acid phosphatase, and bone ash

Source	d.f.	Mean squares				
		Serum phosphatase		Bone phosphatase		Bone ash
		Alkaline	Acid	Alkaline	Acid	percent
Outcome group	5	0.116494	0.024481	1.002786	0.135846	35.37
Replication	1	0.282492	0.066221	0.233289	0.344177	111.30
OCG/Replication	4	0.074994	0.014046	1.195160	0.083763	16.39
Treatment	5	0.699556[a]	0.017514	4.252918[a]	0.412574	6.36
Vit. A vs carotene	1	1.210367[a]	0.026029	0.005576	1.642669	4.34
Levels	2	0.761058[a]	0.004246	5.444980[a]	0.167768	3.28
3,000 vs 1 100,000 + 400,000 IU	1	1.499912[a]	0.005270	2.926184	0.202778	1.87
100,000 vs 400,000 IU	1	0.022204	0.003220	7.963776[a]	0.132759	4.68
Interaction	2	0.382649[b]	0.026524	5.184528[b]	0.042332	10.46
OCG x Treatment	25	0.111981	0.020010	0.948762	0.746413	16.46
Rep. x Treatment	5	0.175928	0.075246	1.293218	1.097480	48.54
OCG/Rep. x Treatment	20	0.095994	0.006201	0.862647	0.658646	8.45
Total	35	0.196565	0.020292	1.428502	0.611498	17.72

[a]Significant at P = .01 or less.

[b]Significant at P = .05 or less.

Table 50. Experiment 6404 - Summary of bone matrix hydroxyproline

Added to diet:						
Vitamin A (IU/lb.)	0	0	0	400,000	100,000	3,000
Carotene (mg/lb.)	750.2	187.3	5.675	0	0	0
Replication	Bone matrix hydroxyproline (%)					
1	2.64	2.64	2.95	3.18	2.41	2.84
2	2.49	2.46	1.91	2.70	2.66	2.49
Av.	2.52	2.55	2.53	2.94	2.54	2.75

Table 51. Experiment 6404 - Analysis of variance for bone matrix hydroxyproline percentage

Source	d.f.	Mean squares
Replication	1	0.3169
Treatment	5	0.0622
Vitamin A vs carotene	1	0.1180
Levels	2	0.0574
3,000 vs 100,000 + 400,000 IU	1	0.0267
100,000 vs 400,000 IU	1	0.0882
Interaction	2	0.0390
Error	5	0.0919
Total	11	0.0988

Table 52. Experiment 6428 - Summary of weight gain at four weeks

Added to diet:						
Vitamin A (IU/lb.)	0	100,000	200,000	300,000	400,000	0
Carotene (mg/lb.)	0	0	0	0	0	938
Replication	Total gain (lb.)					
1	21.5	14.1	12.0	4.7	4.5	10.1
2	22.2	10.9	-5.1	8.0	-1.7[a]	3.3[b]
3	11.2	19.0	10.4	13.2	6.1	7.0
4	11.7	17.0	10.5	7.8	5.1	14.1
Av.	16.6	15.2	7.0	8.4	3.5	8.6

[a]Died after 10 days on experiment.

[b]Died after 24 days on experiment.

Table 53. Experiment 6428 - Analysis of variance for total gain

Source	d.f.	Mean squares
Replication	4	26.42
Treatment	5	102.17
Linear	1	312.07[a]
Quadratic	1	101.31
Cubic	1	29.16
Quartic	1	0.01
Quintic	1	68.05
Error	12	31.08
Total	21	47.12

[a]Significant at P = .01 or less.

Table 54. Experiment 6428 - Summary of plasma hydroxyproline, blood hemoglobin concentration, and hematocrit

Added to diet:						
Vitamin A (IU/lb.)	0	100,000	200,000	300,000	400,000	0
Carotene	0	0	0	0	0	938
Replication						
	Plasma hydroxyproline (mcg/ml)					
1	9.1	8.6	9.8	9.4	13.4	10.0
2	9.3	9.0	10.8	9.1	9.9	10.9
3	8.9	9.8	9.4	9.4	14.4	11.5[a]
4	9.4	10.2	9.2	9.3	9.8	11.8
Av.	9.2	9.4	9.8	9.3	11.9	11.0
	Hemoglobin (gm/100 ml)					
1	9.8	11.1	9.4	8.8	9.4	8.9
2	8.5	9.7	8.1	8.2	8.8	7.8
3	8.5	9.5	8.1	9.5	10.5	6.5[a]
4	5.6	10.2	8.2	9.0	12.1	9.0
Av.	8.1	10.1	8.4	8.9	10.2	8.0
	Hematocrit (%)					
1	33.7	38.9	35.6	30.6	32.4	31.1
2	20.3	39.4	33.3	30.1	31.6	30.1
3	33.3	35.5	30.8	34.0	37.2	32.8[a]
4	29.6	33.3	30.4	31.5	38.7	32.6
Av.	29.2	36.8	32.5	31.6	35.0	31.6

[a]Calculated missing value.

Table 55. Experiment 6428 - Analysis of variance for plasma hydroxyproline, blood hemoglobin, and hematocrit

Source	d.f.	Mean squares		
		Hydroxyproline	Hemoglobin	Hematocrit
Replication	3	0.63	1.21	12.27
Treatment	5	4.90[a]	3.78	29.02
Linear	1	15.18[b]	0.01	1.89
Quadratic	1	0.57	3.75	26.69
Cubic	1	0.79	0.14	18.21
Quartic	1	4.17	14.79[b]	98.25[b]
Quintic	1	3.81	0.23	0.04
Error	14	1.45	1.42	12.36
Total	22	2.12	1.93	16.13

[a]Significant at P = .05 or less.

[b]Significant at P = .01 or less.