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HYPERVITAMINOSIS A IN RABBITS. I. DOSE RESPONSE

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Summary

Sixty-four 5 week-old New Zealand White rabbits were assigned to eight treatments, which consisted of vitamin A (retinol palmitate) administered orally daily at the following levels: 0 (control), 3,000, 6,000, 12,000, 30,000, 60,000, 90,000 and 120,000 IU/animal/day. Body weight and feed intake were recorded weekly. At the end of the experimental period, four rabbits from each group were sacrificed to collect liver and blood samples for vitamin A analyses and assays of plasma Ca, P and glucose. Significant decreases in body weight, retardation of growth and significant reductions in feed intake were observed with the higher doses. Plasma and liver vitamin A levels increased significantly, by about 2 and 60 fold, respectively, with higher doses of vitamin A. Differences in plasma Ca and glucose were not significant. There was a slight increase in plasma P with the higher doses of vitamin A.

Introduction

Vitamin A has toxic and teratogenic effects when given in excess to animals. Vitamin A hypervitaminosis has often been referred to as a universal teratogenic procedure. The toxicity effect of vitamin A depends on dose, species, animal size, age, method and period of administration (Nieman and Obbink, 1954), nutritional status, factors involved in the absorption of fat-soluble vitamins (Holander, 1981), disease state, nutritional interaction (Veltmann and Jensen, 1986) and the chemical form of vitamin A. Retinol is the most potent teratogen (James, 1976; Cheeke, 1987).

Growth rate and cellular differentiation depend upon the level of vitamin A. The effects of high vitamin A on growth performance of rabbits and other laboratory animals have been well established (Chew and Archer, 1983; Kormann and Schlachter, 1984; Elmarimi et al, 1989). Studies in large animals have shown similar results, where the signs of hypervitaminosis A were arrested growth, loss in weight due to reduced appetite (anorexia) and impaired bone development (Nieman and Obbink, 1954; Cheeke, 1987; McDowell, 1989).

The objective of this study was to determine the toxic levels of synthetic vitamin A when added to a high-alfalfa diet rich in carotene, and to determine the relationship between blood and liver vitamin A levels under such conditions.

Materials and Methods

A total of 64 New Zealand White rabbits about 5 weeks of age and 600 g average body weight were used. The animals were randomly placed in individual wire cages. A standard OSU diet (Table 1) and fresh drinking water were provided ad libitum. Eight rabbits were randomly assigned to each of the eight treatments, which consisted of vitamin A administered orally daily as retinol palmitate at the following levels: 0 (Control), 3,000, 6,000, 12,000, 30,000, 60,000, 90,000 and 120,000 IU/animal/day (treatments 1, 2, 3, 4, 5, 6, 7 and 8, respectively).

Table 1. Composition of the standard OSU diet.

Ingredient	% of Mix
Alfalfa	56.54
Wheat mill run	36.99
Molasses	3.00
Bentonite	1.25
Copper sulfate	0.10
Vitamin A*	0.60
Vitamin E**	0.20
Salt	0.50
Meat meal	0.819

* Provided 13,200 IU vitamin/kg diet.

** Provided 22 IU vitamin E/kg diet.

Body weight and feed intake were recorded weekly (5 weeks). At the end of the experimental period, four rabbits from each group were sacrificed to collect liver and blood samples for vitamin A analyses. The remaining blood samples were used for Ca, P and glucose assays. The blood was centrifuged and the plasma prepared. Plasma and liver samples were frozen until analysis.

For vitamin A analysis of plasma and liver, a high performance liquid chromatography (HPLC) method was used (Bieri et al., 1974; Chow et al., 1983; Omaye et al., 1986). For calcium determination, an atomic absorption spectrophotometry method was used (Thomas, 1976). Phosphorus in the plasma and diet was determined spectrophotometrically at 470 nm according to Thomas (1976). Glucose in plasma was assayed by enzymatic (hexokinase) method and measured spectrophotometrically at 340 nm.

Statistical analysis was conducted by analysis of variance using the SAS Package (1990). The means and standard error of all studied parameters were estimated and Tukey's test was used to detect significant differences among the means of the experimental groups. Correlation analyses between level of vitamin A and body weight and feed intake and also between vitamin A in the liver and plasma were conducted.

Results and Discussion

Body weight, body gain and feed intake

Mean weekly body weight (± SE), total body gain and mean weekly feed intake are shown in Table 2 and Figures 1, 2 and 3, respectively. The high vitamin A levels had a severe effect on body weight and this effect was obvious starting from the first week of treatment. There was a significant ($P<0.001$) decrease in body weight especially in treatment 8 (120,000 IU/animal/day) compared to other treatments. From the 2nd week until the end of the experiments, differences between treatments increased significantly from $P<0.001$ to $P<0.0001$, indicating the cumulative effect of vitamin A. Body weight was significantly higher in treatments 1, 2, 3, 4 and 5 than in 6, 7 and 8 from weeks 2 to 5 (see Table 2 and Figure 1). A similar trend was observed for total body gain (Figure 2). This value was much higher in treatments 1, 2 and 3, relatively higher in treatments 4 and 5, without any significant differences among the 5 treatments, and very low in treatments 6, 7 and 8, with the lowest value 619.8 g.

Hypervitaminosis A reduced feed intake, and differences between treatments were statistically significant ($P<0.001$) starting from the 2nd week and continuing until the end of the experimental period. Tukey's test revealed that feed intake was drastically lower in treatment 8 compared to all other treatments throughout the experimental period. Results indicated that vitamin A reduced body weight, total body gain and feed intake, with a negative correlation between the dose of vitamin A and body weight from the 1st week and feed intake from the 2nd week. The correlation increased gradually from -0.42 to -0.51, -0.62, -0.65 and -0.74 for body weight, and from 0.09 to -0.58, -0.52, -0.54 and -0.74 for feed intake in the 1st, 2nd, 3rd, 4th and 5th week, respectively. Similar results were reported by Rodahl (1950), Hilton (1983), Cheeke et al. (1984) and McDowell (1989).

Table 2. Means ± SE for body weight, body gain and feed intake.

IU Vitamin A Per Animal Per Day	Body Weight (g)					Body Gain (g)	Feed Intake (g)				
	1st week	2nd week	3rd week	4th week	5th week		1st week	2nd week	3rd week	4th week	5th week
Diet 1. 0	966.5 ± 66.74^{a}	1204.25 ± 80.65^{a}	1435 ± 80.02^{a}	1599.75 ± 83.45^{a}	1891.13 ± 81.84^{a}	1222.88	681.62 ± 11.82^{a}	873.37 ± 53.81^{a}	929.62 ± 40.12^{a}	1003.87 ± 22.15^{a}	1192 ± 45.97^{ab}
Diet 2. 3,000	939.25 ± 66.85^{a}	1097.88 ± 78.44^{abc}	1313.13 ± 87.32^{abc}	1505 ± 80.70^{abc}	1836.88 ± 96.23^{a}	1192.0	653 ± 19.02^{a}	761.62 ± 41.85^{a}	898.5 21.41^{a}	1018.12 45.84^{a}	1280.25 60.02^{a}
Diet 3. 6,000	940.38 ± 71.57^{a}	1145 ± 80.54^{abc}	1395.86 ± 67.93^{ab}	1557.43 ± 58.81^{ab}	1887.71 ± 68.83^{a}	1252.33	725.25 ± 22.95^{a}	862.75 ± 39.66^{a}	915.57 ± 35.42^{a}	1039.57 ± 39.53^{a}	1217 ± 32.53^{ab}
Diet 4. 12,000	904.13 ± 70.85^{ab}	1055.75 ± 91.6^{abc}	1282.29 ± 76.17^{abc}	1468.57 ± 69.04^{abc}	1721 ± 65.88^{ab}	1067.37	716.37 ± 18.28^{a}	803.25 ± 55.15^{a}	905 ± 45.24^{a}	1000 ± 47.49^{a}	1011.14 ± 37.89^{bcd}
Diet 5. 30,000	912.63 ± 61.27^{a}	1018.14 a ± 85.55^{abc}	1223 ± 106.33^{abcd}	1447 ± 100.75^{abc}	1708.17 ± 84.45^{abc}	1033.17	706.50 ± 16.55^{a}	710.29 ± 30.20^{ab}	931.83 ± 40.06^{a}	955.5 ± 65.47^{ab}	1105.33 ± 78.45^{abc}
Diet 6. 60,000	812.75 ± 56.83^{ab}	980 ± 44.90^{abc}	1087.17 ± 39.56^{cd}	1232.5 ± 53.18^{cd}	1407.17 ± 64.06^{cd}	763.17	688.37 ± 31.37^{a}	740.86 ± 26.77^{a}	822.17 ± 42.11^{a}	851.17 ± 49.65^{ab}	903 ± 78.69^{cd}
Diet 7. 90,000	793.63 ± 38.21^{ab}	937.63 ± 32.20^{bc}	1118.25 ± 29.8^{bcd}	1263.5 ± 33.42^{bcd}	1453.13 ± 26.09^{bcd}	843.5	710.25 ± 11.98^{a}	696.37 ± 12.19^{ab}	845.5 ± 24.9^{a}	929.25 ± 28.58^{ab}	885 ± 33.18^{cd}
Diet 8. 120,000	711 ± 62.55^{b}	772.38 ± 84.33^{b}	931.57 ± 87.54^{d}	1050.14 ± 87.57^{d}	1211.43 ± 82.56^{d}	619.8	687.25 ± 26.18^{a}	542 ± 46.96^{b}	686.86 ± 32.16^{b}	772.43 ± 31.62^{b}	798.57 ± 23.82^{d}

Within each column any two means having at least one similar letter are not significantly different.

FIG. 1. EFFECT OF DIFFERENT LEVELS OF VITAMIN A ON BODY WEIGHT

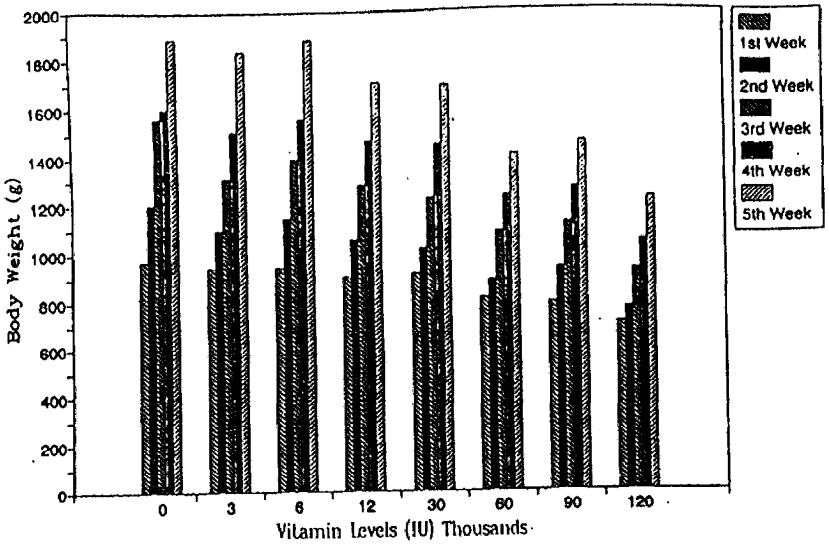


FIG. 2. EFFECT OF DIFFERENT LEVELS OF VITAMIN A ON BODY GAIN

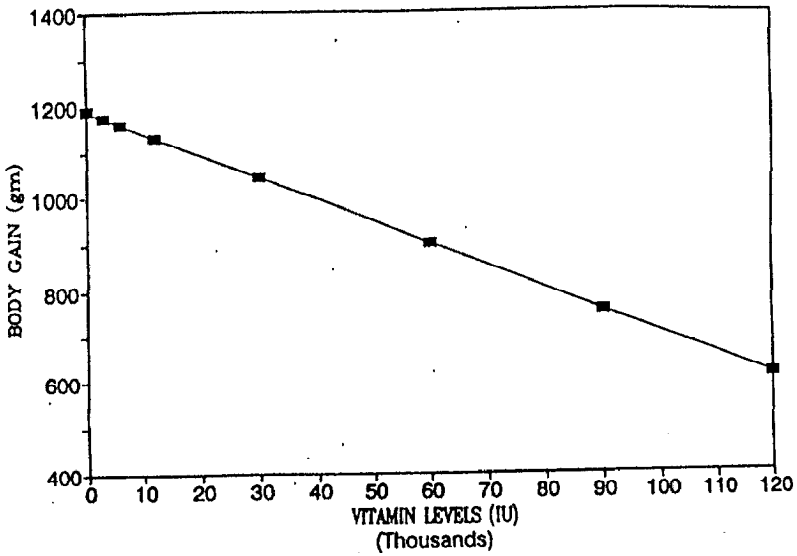
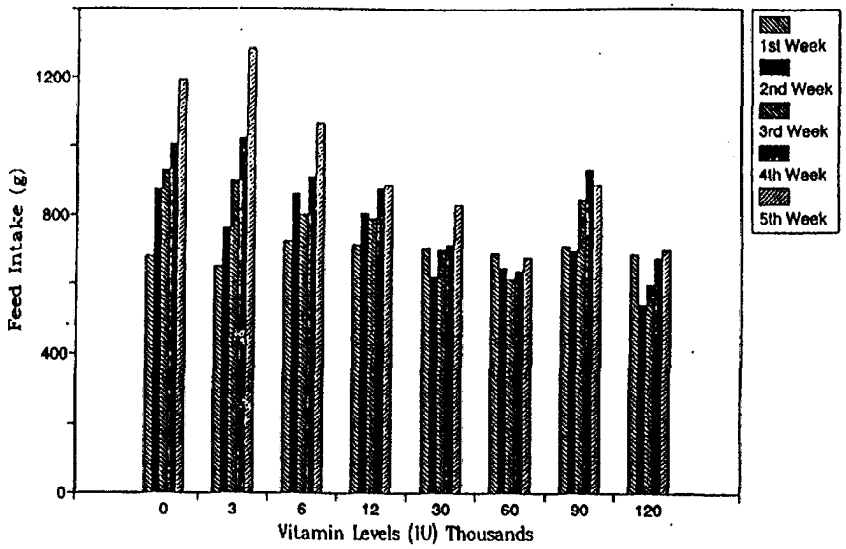


FIG. 3. EFFECT OF DIFFERENT LEVELS OF VITAMIN A ON FEED INTAKE



Overdoses of vitamin A result in arrest of growth due to loss of appetite (anorexia), reduced feed intake, retardation of growth, loss in weight, emaciation accompanied by anemia, and abnormal bone development. The effect of vitamin A on growth is mediated by many factors such as dose, administration method and period, vitamin form, species, age, animal size and initial weight, nutritional status, factors involved in the absorption of fat-soluble vitamins, disease and nutrition interaction (James, 1976; Holander, 1981; Veltmann and Jensen, 1986). In this study, animals smaller in size or lower in weight were more sensitive and susceptible to vitamin A overdose.

Average plasma and liver vitamin A levels are shown in Table 3. Differences in vitamin A levels in the plasma and liver due to treatments were significant ($P < 0.01$). The plasma vitamin A level increased with increasing oral dose, especially with high toxicity levels of vitamin A (groups 7 and 8). The levels were significantly higher in groups 7 and 8 than in the others (see Table 3). Thus, plasma vitamin A is a good indicator of severe vitamin A toxicity or deficiency (Bondi and Sklan, 1984). Rabbits receiving 120,000 IU/animal/day had only a two-fold increase in plasma level vs. a 60-fold increase in liver; thus liver vitamin A concentration is the best indicator of vitamin A status, clearly reflecting the dietary level of vitamin A. A highly significant increase in liver vitamin A was noted in treatment 2 as compared to the control group, with a concentration 5 times the level in treatment 1. The concentration increased gradually with increasing oral dose until group 8 where the concentration was 60-fold the control group. In this study, Tukey's test revealed highly significant differences between all experimental levels used except between treatment 2 and 3 (3000 and 6000 IU/animal/day, respectively). There was a positive correlation (0.77) between liver and plasma vitamin A concentrations. The present results are in agreement with those of Bondi and Sklan (1984), Cheeke et al. (1984), Moghaddam et al. (1987), Elmarimi et al. (1989) and Deeb et al. (1992).

Average plasma Ca, P and glucose are shown in Table 3. Abnormal bone formation, indicated by bone fragility, was noted especially in treatments 7 and 8, suggesting that overdosage of vitamin A might affect Ca and P metabolism and plasma levels. Differences in plasma Ca due to treatments were not significant and values were higher than the normal value including the control group (19.05 ± 1.87 mg/100 ml vs. the normal value of 10 mg/100 ml). The dietary Ca level was 0.9% (the recommended level is 0.4%; NRC, 1977). The plasma Ca level in rabbits is not regulated homostatically as in other species, but varies in direct proportion to dietary Ca level, and the absorption rate of Ca is not regulated to meet the metabolic need for Ca (Cheeke, 1987). Thus, in this study, high dietary Ca masked the effect of vitamin A on plasma Ca. Dietary P was higher than the recommended level (0.6% vs. 0.3%; NRC, 1977). Thus, plasma P was higher in the control group (8.23) than the normal value (6 mg/100 ml) according to Barlet (1980) and in the normal range according to Cheeke et al. (1985). Tukey's test indicated that there is a significant increase in plasma P in treatments 3, 4, 5, 6, 7, and 8 as compared to treatments 1 and 2. The role of vitamin A in normal development of bone is control over the activity of osteoclasts and osteoblasts of the epithelial cartilage

(Mellanby, 1947; McDowell, 1989) and the pathologic effect is increased activity of osteoclasts or decreased activity of osteoblasts while the activity of osteoclasts remains unaffected (Irving, 1949). In both cases, longitudinal growth is accelerated more than lateral growth. This is the way that bones become fragile (Nieman and Obbink, 1954; Kai-Ning et al., 1985). Disturbed bone formation and bone fragility, as noticed in this study, without any change in Ca and P content of the plasma or with a slight hypocalcemia and hyperphosphatemia in rabbits were reported by Rodahl (1950), Nieman and Obbinsk (1954) and Veltmann and Jensen (1986).

Table 3. Means ± SE for vitamin A in liver and vitamin A, Ca, P, and glucose in plasma.

	Blood Plasma				
Treat-ments	Ca, mg/ 100 ml	P, mg/ 100 ml	Glucose, mg/dl	Vitamin A, IU/ml	Liver Vitamin A, IU/g
1	19.05 ± 1.87^{a}	8.23 ± 0.18^{c}	107.99 ± 11.66^{a}	3.72 ± 0.33^{c}	210.06 ± 28.33^{g}
2	18.98 ± 1.68^{a}	8.04 ± 0.41^{c}	81.41 ± 9.14^{a}	4.02 ± .28^{c}	1045.88 ± 128.54^{f}
3	19.13 ± 1.98^{a}	9.39 ± 0.35^{ab}	93.76 ± 6.39^{a}	3.91 ± 0.33^{c}	1563.92 ± 143.47^{f}
4	17.29 ± 0.94^{a}	8.97 ± 0.44^{b}	91.27 ± 13.4^{a}	4.11 ± 0.29^{c}	3140.16 ± 287.57^{e}
5	19.84 ± 0.50^{a}	10.23 ± 0.22^{a}	96.08 ± 7.27^{a}	4.83 ± .62^{bc}	5034.4 ± 190.83^{d}
6	20.10 ± 1.20^{a}	9.30 ± 0.38^{ab}	100.23 ± 4.27^{a}	5.10 ± 0.62^{bc}	5782.38 ± 216.44^{c}
7	18.30 ± 1.26^{a}	8.96 ± 0.21^{b}	96.94 ± 3.75^{a}	5.74 ± 0.33^{ab}	8157.69 ± 371.64^{b}
8	18.79 ± 0.42^{a}	8.99 ± 0.38^{b}	99.03 ± 5.38^{a}	6.48 ± 0.43^{a}	11380.64 ± 297.35^{a}

Overall means with the same letter are not significant.

Vitamin A has a role in the function of the pancreatic islets of Langerhans, which are important factors in the regulation of carbohydrate metabolism (Sporn et al., 1984; Wolfe, 1984). Thus it is assumed that an overdose of vitamin A might affect carbohydrate metabolism, insulin secretion, and, subsequently, plasma glucose. Differences in plasma glucose due to treatments were not significant. This was unexpected as an overdose of vitamin A inhibits secretion and/or release of insulin and subsequent increase in plasma glucose (hyperglycemia). The non-significant differences in plasma glucose may have been due to a specific sensitivity for insulin action in rabbits, or perhaps were due to the dosage of vitamin A or short experimental period. In agreement with these results, Bardos and Pusztai (1989) showed that plasma glucose was

unaffected by peroral administration of 50,000 IU vitamin A, and the blood glucose level remained relatively steady in rabbits treated with 100,000 IU vitamin A/animal and 1 g glucose/kg body weight. Shankar and De-luca (1988) and Shankar et al. (1990) failed to detect any changes in blood glucose in a 7 week study, and decided that a 9 week period would be necessary to detect blood glucose changes.

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References

Bardos, L. and A. Pusztai. 1989. Effect of peroral administration of vitamin A and glucose on blood sugar and insulin levels of rabbits. J. Appl. Rabbit Res. 12:247-249.

Barlet, J.P. 1980. Plasma calcium, inorganic phosphorus and magnesium levels in pregnant and lactating rabbits. Reprod. Nutr. Dev. 20:647-651.

Bieri, J.G., T.J. Tolliver and G.L. Catignani. 1979. Simultaneous determination of α-tocopherol and retinol in plasma or red cells by high pressure liquid chromatography. Am. J. Clin. Nutr. 32:2143.

Bondi, A., and D. Sklan. 1984. Vitamin A and carotene in animal nutrition. Prog. Food Nutr. Sci. 8:165-191.

Cheeke, P.R., N.M. Patton, K. Diwyanto, A. Lasmini, A. Nurhadi, S. Prawirodigdo and B. Sudaryanto. 1984. The effect of high dietary vitamin A levels on reproductive performance of female rabbits. J. Appl. Rabbit Res. 7:135-137.

Cheeke, P.R., J. Bronson, K.L. Robinson and N.M. Patton. 1985. Availability of calcium, phosphorus and magnesium in rabbit feeds and mineral supplements. J. Appl. Rabbit Res. 8:72-74.

Cheeke, P.R. 1987. Rabbit Feeding and Nutrition. Academic Press, San Diego.

Chew, B.P. and R.G. Archer. 1983. Comparative role of vitamin A and β-carotene on reproduction and neonate survival in rats. Theriogenology 20:459-472.

Chow, F.I. and S.T. Omaye. 1983. Use of antioxidants in the analysis of vitamin A and E in mammalian plasma by high performance liquid chromatography. Lipids 18:837.

Deeb, B.J., R.F. DiGiacomo and R.J. Anderson. 1992. Reproductive abnormalities in rabbits due to vitamin A toxicity. J. Appl. Rabbit Res. 15:974-985.

Elmarimi, A.A., E. Ven and L. Bardos. 1989. Preliminary study on the effects of vitamin A and β-Carotene on growth and reproduction of female rabbits. J. Appl. Rabbit Res. 12:163-168.

Hilton, J.W. 1983. Hypervitaminosis A in rainbow trout (Salmo gairdneri). Toxicity signs and maximum tolerable level. J Nutr. 113:1737-1745.

Holander, D. 1981. Intestinal absorption of vitamins A, E, D, K. J. Lab. Clin. Med. 97:449-462.

Irving, J.T. 1949. The effects of A avitaminosis and hypervitaminosis A upon the incisor teeth and incisal alveolar bone of rats. J. Physiol. (London) 108:92-101.

James, S.L. 1976. Drugs as teratogens. Cleveland, CRC Press.

Kai-Ning, T., G.N. Rowland and J.R. Veltmann. 1985. Vitamin A toxicity: comparative changes in bone of the broiler and leghorn chicks. Avian Diseases 29:416-429.

Kormann, A.W. and M. Schlachter. 1984. Preliminary trials concerning growth and reproduction of rabbits on variable supplementation of β-carotene and vitamin A. Proceedings, III World Rabbit Congress, Rome, Italy.

McDowell, L.R. 1989. Vitamins in animal nutrition. Academic Press, Inc, San Diego.

Mellanby, E. 1947. Vitamin A and bone growth: The reversibility of vitamin A deficiency changes. J. Physiol. (London) 105:382-399.

Moghaddam, M.F., P.R. Cheeke and N.M. Patton. 1987. Toxic effect of vitamin A on reproduction in female rabbits. J. Appl. Rabbit Res. 10:65-67.

Nieman, C. and H.J.K. Obbink. 1954. The biochemistry and pathology of hypervitaminosis A. Vitamins and Hormones 12:69-99.

NRC. 1977. Nutrient requirements of domestic animals, nutrient requirements of rabbits, 2nd ed. National Academy of Science, National Research Council, Washington, D.C.

Omaye, S.T., and F.I. Chow. 1986. Distribution of vitamin A and E in blood and liver of rats depleted of vitamin A or vitamin E. Lipids 21:465.

Rodahl, K. 1950. Hypervitaminosis A in the rat. J. Nutrition 41:399-421.

SAS Institute, Inc. 1990. SAS/STAT Guide for Personal Computers, version 6.03 ed. SAS Institute, Inc., Cary, N.C.

Shankar, S. and L. De-luca. 1988. Retinoic acid supplementation of vitamin A deficiency diet inhibits retinoid loss from hamster liver and serum pools. J. Nutr. 8:675-680.

Shankar, S., K.E. Creek and L. De-luca. 1990. The effect of the progression of vitamin A deficiency on glucose, galactose and mannose incorporation into sugar phosphates and sugar nucleotides in hamster liver. J. Nutr. 120:361-374.

Sporn, B.M., B.A. Roberts and D.S. Goodman. 1984. The retinoids I-JI. Academic Press, New York.

Thomas, G. 1976. Chemical analysis of plant tissue. Search Agriculture, Vol. 6 No. 8.

Veltmann, J.R., Jr., and L.S. Jensen. 1986. Vitamin A toxicosis in the chick and turkey poults. Poult. Sci. 65:538-545.

Wolf, G. 1984. Multiple functions of vitamin A. Physiol. Rev. 64:873.