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HYPERVITAMINOSIS A IN RABBITS. II. INTERACTIONS WITH VITAMINS E AND C AND ETHOXYQUIN

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Summary

Forty New Zealand White rabbits 56 days of age were assigned to five groups, which consisted of orally daily administered vitamins A (retinol palmitate), C (L-ascorbic acid) and E (α-tocopherol) and ethoxyquin as follows: 0 supplementation (control), 12,000 IU vitamin A, 12,000 IU vitamin A + 24 mg vitamin C, 12,000 IU vitamin A + 24 mg vitamin E and 12,000 IU vitamin A + 12 mg ethoxyquin/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 plasma Ca, P and glucose were assayed. There were no significant differences in body weight or feed intake between all the experimental groups. Plasma and liver vitamin A were elevated in all experimental groups as compared to the control group and the differences were significant. Vitamin E and ethoxyquin may play an important role in vitamin A storage in the liver. Differences in plasma Ca and glucose were not significant. There was a significant increase in plasma P in groups A+C, A+E and A + ethoxyquin as compared to group A and the control group. The effects of overdoses of vitamin A on plasma Ca and P alone or in combination with vitamins C and E and ethoxyquin were not great, ranging from no change to slight hypocalcemia and hyperphosphatemia, respectively.

Introduction

Although vitamins are essential in the diet for normal health, some of them have adverse physiological effects when consumed in excessive amounts. It is important to understand the range of tolerance for each vitamin and the biochemical consequences of consumption of large quantities of these vitamins, in particular with regard to their interaction with one another and with other nutrients. Vitamin A is essential for vision, growth and cellular differentiation. The effects of vitamin A on different physiological functions in different species have been well established (McDowell, 1989).

Rabbits have a very rapid growth rate during the first three months of life. Rapid growth makes these animals very sensitive to non-specific syndrome, because of the rate of metabolism and cell division which is very high compared to that of other mammals. During this period of rapid growth, any nutrient deficiency or overdose is likely to have a major effect (Hannan, 1971; White et al., 1983).

Biological membranes contain relatively high concentrations of polyunsaturated fatty acids, making them susceptible to lipid peroxidation (Tappel, 1962), and it is important to use antioxidants to protect the lipid components against oxidation. Bartov and Bornstein (1972) reported that antioxidants act in a manner similar to vitamin E. Combs and Regenstein (1980) supported this hypothesis by demonstrating a protective effect of ethoxyquin against rancidity.

The disturbance of physiological equilibrium between vitamin A and other factors, caused by hypervitaminosis, illustrates the normal function of vitamin A in the body (Nieman and Obbink, 1954). The interrelationship between excessive vitamin A and other nutrients has not been systematically studied. The objective of this study was to determine if the absorption and blood and liver levels of vitamin A in rabbits are influenced by oral administration of vitamins C, E or ethoxyquin, a synthetic antioxidant.

Materials and Methods

Forty New Zealand White rabbits of about 56 days of age and 900 g average body weight were assigned to five experimental groups. Eight rabbits were randomly placed in individual wire cages for each treatment. A standard OSU diet (Table 1) and water were available ad libitum. Treatments consisted of orally administered vitamin A (retinol palmitate), vitamin C (L ascorbic acid) and vitamin E as a natural antioxidant and ethoxyquin as a synthetic antioxidant as follows: 0 supplementation (control), 12,000 IU vitamin A/animal/day, 12,000 IU vitamin A + 24 mg vitamin C/animal/day, 12,000 IU vitamin A + 24 mg vitamin E and 12,000 IU vitamin A + 12 mg ethoxyquin/animal /day for treatments 1, 2, 3, 4 and 5, respectively.

Body weight and feed intake were recorded weekly during the experimental period (6 weeks). At the end of the experimental period, four rabbits from each group were sacrificed to collect liver and blood plasma for vitamin A analysis. Plasma calcium, phosphorus and glucose were assayed. The blood was centrifuged, plasma prepared and plasma and liver were frozen until analysis. For all analyses, the same techniques and procedures were used as in the previous experiment (Ismail et al., 1992a). Statistical analysis was conducted similarly to that of the previous work (Ismail et al., 1992a).

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	0.82

* Provided 13,200 IU vitamin A/kg diet.

** Provided 22 IU vitamin E/kg diet.

Results and Discussion

Body Weight, Total Body Gain and Feed Intake

Average weekly body weight (± SE), total body gain and average weekly feed intake (± SE) are shown in Table 2. There were no significant differences in body weight or feed intake between all the experimental groups including the control group. This result can be attributed to the vitamin A dosage used in this study (12,000 IU/animal/day), which would be a good level for growing rabbits (Ismail et al., 1992a). No sign of vitamin A toxicity was detected in growth rate or feed intake. This result may also have been due to the high initial body weight (900 g) which enabled the animals to tolerate this level of vitamin A. Unfortunately, the vitamin A level used and the high initial body weight masked the effect of vitamin A and the interaction between vitamin A and vitamins E, C and ethoxyquin.

The present results are in agreement with studies of Harris et al. (1983), Grobner et al. (1985), Kormann and Schlater (1984), Elmarimi et al. (1989) and Ismail et al. (1992a), in which no significant differences between control and diets supplemented with various vitamins on daily feed intake or gain were noted. These results tend to agree with Cabel et al. (1988) who reported that the addition of ethoxyquin had little or no

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

	Body Weight (g)							Feed Intake (g)					
Treat-ment	1st Week	2nd Week	3rd Week	4th Week	5th Week	6th Week	Body Gain (g)	1st Week	2nd Week	3rd Week	4th Week	5th Week	6th Week
1	1190.38 ± 69.30	1388.57 ± 61.90	1657.75 ± 52.70	1934.13 ± 71.48	2206.38 ± 59.78	2432.8 ± 74.14	1538.17	708.62 ± 43.11	884.12 ± 45.51	957.25 ± 25.94	1070.00 ± 46.55	1282.88 ± 40.15	1328.75 47.92
2	1145.50 ± 61.12	1309.25 ± 52.86	1557.00 ± 53.67	1810.63 ± 67.32	2101.25 ± 70.89	2290.1 ± 91.53	1398.38	652.75 ± 41.97	773.87 ± 31.39	961.62 ± 28.53	1091.25 ± 58.69	1265.00 ± 51.49	1278.13 ± 41.50
3	1138.75 ± 43.16	1306.63 ± 37.97	1577.63 ± 29.93	1892.13 ± 49.95	2128.63 ± 55.36	2321.3 ± 65.91	1427.55	671.12 ± 40.90	839.50 ± 12.43	952.00 ± 30.53	1148.13 ± 34.02	1230.75 ± 66.55	1207.50 ± 43.85
4	1176.25 ± 50.17	1383.79 ± 59.33	1616.38 ± 81.62	1895.50 ± 76.99	2177.63 ± 62.75	2383.4 ± 65.82	1469.65	709.12 ± 39.12 ±	891.37 34.38	919.62 ± 58.42 ±	1123.75 21.77	1285.88 ± 41.95	1268.75 ± 45.69
5	1183.00 ± 52.17	1415.57 ± 33.90	1704.29 ± 51.41	1965.14 ± 44.75	2277.14 ± 37.24	2529.7 ± 49.87	1616.96	650.37 ± 45.22	860.86 ± 51.45	1002.29 ± 37.93	1191.43 ± 40.79	1354.29 ± 21.70	1391.43 ± 28.65

effect on either body weight or feed efficiency in broilers. However, in our study, group 5 (A + ethoxyquin) showed a slight increase in body weight (2529.7) compared to group 2 (A; 2290.1). The same trend was observed in total body gain, which was 1616.96 for group 5 and 1398.38 for group 2.

The mode of action of ethoxyquin is not fully understood, but it seems to improve animal performance by inhibiting the deleterious effect of peroxide and rancid diets by preventing continual peroxide formation or products of oxidation or both, or it may block the physiological or biochemical point at which peroxide or oxidation products or both exert their negative effect (Cabel et al., 1988).

Plasma and Liver Vitamin A

Average plasma and liver vitamin A levels are shown in Table 3. Plasma and liver vitamin A were elevated in all experimental groups as compared to the control group and the differences were highly significant ($P<0.01$ and $P<0.0001$ for plasma and liver, respectively). Tukey's test revealed that there were no significant differences in plasma level between group 4 (A+E) and group 1 (control), which might have been due to an antagonistic interaction between vitamins A and E (such an effect is clear in reproduction, where vitamin E counteracts vitamin A toxicity). Although vitamin E acts as a natural antioxidant towards fat-soluble vitamins and preserves vitamin A activity, it is not as effective as ethoxyquin in preventing rancidity of fat soluble vitamins. There was a significant increase in plasma vitamin A in group 2 (A), group 3 (A+C) and group 5 (A + ethoxyquin) as compared to group 1 (control). The highest value was recorded in group 5 (6.05 ± 0.35), due to the effectiveness of ethoxyquin as a synthetic antioxidant in preventing the autoxidation process that causes rancidity of fat soluble vitamins. Ethoxyquin helps maintain vitamin A activity in plasma and liver (Isenstein, 1970; Cabel et al., 1988). In group 3 (A+C) the plasma vitamin A level was high in spite of antagonistic interaction between vitamin A and ascorbic acid. This antagonism was reported by Niemann and Obbink (1954), who found that ascorbic acid had a marked effect in delaying the deposit of vitamin A in liver. Present results are in agreement with previous conclusions and explain the high level of vitamin A in plasma and the low level in liver in group 3 (A + C) (see Table 3).

Vitamin E and ethoxyquin may play an important role in vitamin A storage in the liver, whereas liver vitamin A concentration in group 4 (A+E) and group 5 (A + ethoxyquin) were higher (2740.85 ± 336.83 and 2723.66 ± 256.74) than in group 2 (A) (2335.07 ± 269.24) or group 3 (A+C) which had the lowest value 1876.91 ± 346.04. Despite the lack of significant differences between treated groups, the effect of vitamin E and ethoxyquin in keeping vitamin activity or storage in the liver was detected. Correlation between plasma and liver vitamin A was low (0.39) and not significant,

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	Vit. A, IU/ml	Liver Vitamin A, IU/g
1	16.24 ± 0.95	6.79 ± 0.61^{b}	105.43 ± 10.68	3.88 ± 0.28^{c}	207 ± 25.30^{b}
2	16.65 ± 1.79	6.26 ± 0.75^{b}	103.49 ± 3.40	5.87 ± 0.19^{a}	2335.07 ± 269.24^{a}
3	15.08 ± 0.94	9.26 ± 0.47^{a}	99.11 ± 7.75	5.26 ± 0.50^{ab}	1876.91 ± 346.04^{a}
4	16.35 ± 1.47	8.73 ± 0.28^{a}	89.62 ± 3.91	4.36 ± 0.29^{bc}	2740.85 ± 336.83^{a}
5	16.84 ± .86	9.26 ± 0.33^{a}	87.71 ± 5.19	6.05 ± 0.35^{a}	2723.66 ± 256.74^{a}

Overall means with the same letter are not significant.

perhaps due to the dosage used (12,000 IU/animal/day). According to Ismail et al. (1992a), this dose would be appropriate for growing rabbits and would not be toxic. A high correlation between plasma and liver vitamin A values is seen only in severe toxicity or deficiency (Bondi and Sklan, 1984). Also, liver vitamin A concentration was found to be affected by body weight. Young animals with lesser body weight were more sensitive and had higher liver vitamin A concentrations (3140.16 ± 287.57) (Ismail et al., 1992a) compared to heavier animals in the present study given the same dose (12,000 IU/animal/day) (2335.07 ± 26.92). Present results are in agreement with those of Bondi and Sklan (1984), Cheeke et al. (1984), Moghaddam et al. (1987), Elmarimi et al. (1989), Deeb et al. (1992), and Ismail et al. (1992a).

Plasma Ca, P and Glucose

Average plasma Ca, P and glucose are shown in Table 3. There were no significant differences in plasma Ca and the effect of 12,000 IU/animal/day vitamin A alone or in combination with vitamins E and C and ethoxyquin is not clear from this study. The vitamin A dosage may not have been suitable for determining an effect on plasma minerals. Also, the high dietary calcium level (0.9%) may have obscured an interaction. Plasma Ca level in rabbits is not regulated homostatically, but varies in direct proportion to dietary Ca (Cheeke, 1987). Thus it was difficult to detect any differences between treatments and the control group, or to determine whether results were within normal range or higher than normal, due to contradictory values obtained previously (10 mg/100 ml to 16.8 mg/100 ml). Plasma Ca seemed to be affected by age and body weight, being higher in young rabbits (19.05 ± 1.87 in the control group [Ismail et al., 1992a] than in older and heavier rabbits in the present study (16.24 ± 0.95 in the control group).

From the present study and previous results, it appears that overdoses of vitamin A have no effect on plasma Ca, alone or in combination with vitamins C and E and ethoxyquin. Effects ranged from no change to slight hypocalcemia in spite of the antagonistic effect of

excessive vitamin A on the activity of vitamin D and subsequent mineral metabolism. These results are in agreement with those of Rodahl (1950b), Nieman and Obbink (1954), Kai Ning et al. (1985), Cheeke et al. (1985), Veltmann and Jensen (1986) and Cheeke (1987).

In contrast to the plasma Ca results, plasma P results showed a significant difference between treatments, and Tukey's test revealed a significant increase in plasma P in group 3 (A+C), group 4 (A+E) and group 5 (A + ethoxyquin) as compared to groups 1 (control) and 2 (A) that showed no difference.

From the present study and previous results, it appears that plasma P is sensitive to overdoses of vitamin A alone or in combination with antioxidant agents. Vitamin E and ethoxyquin increase the effect of vitamin A on plasma P. Possibly the effects of these antioxidants on minerals are mediated through a vitamin A-vitamin D interaction.

Plasma P seemed to be affected by age and body weight. Whereas young rabbits were more affected and showed hyperphosphatemia (8.23 and 8.94 in the control and vitamin A [12000/animal/day] supplemented groups, respectively; Ismail et al., 1992a), the older and heavier animals in the present study were not affected (6.79 and 6.26 for the control and vitamin A [12000/animal/day] supplemented groups, respectively). Young rabbits also showed hyperphosphatemia (9.39) when treated with 6000 IU vitamin A/animal/day (Ismail et al., 1992a), but in this study, our animals showed hyperphosphatemia when treated with a high dose of vitamin A (12,000/animal/day) combined with antioxidant factors (C, E and ethoxyquin), not alone.

The effect of overdoses of vitamin A on plasma P alone or in combination with vitamins C and E and ethoxyquin was not great, ranging from no change to slight hyperphosphatemia. The role of vitamin C in hyperphosphatemia is not clear in spite of the antagonistic interaction between excess vitamin A and ascorbic acid, with ascorbate reducing the adverse effects of high vitamin A on bone and the organic matrix of bones and teeth (McDowell, 1989).

Plasma glucose level was unaffected by administration of 12,000 IU vitamin A/animal/day alone or in combination with vitamins E and C and ethoxyquin; differences between groups were not significant.

The effect of vitamin A on plasma glucose has been reported by many workers. High doses of vitamin A inhibit insulin secretion and/or its release from the beta cells of Langerhans islets (Bardos and Pusztai, 1989). To detect the effect of vitamin A on plasma glucose, the experimental period should be longer than 9 weeks, because blood glucose is maintained by regulatory mechanisms. It is more accurate to measure the insulin peak because it is directly affected by the level of vitamin A and is a better indicator than blood glucose, especially in rabbits, which have a specific sensitivity to insulin action (Bardos and Pusztai, 1989). Shankar and De-luca (1988) reported that, in a case of vitamin A deficiency, blood glucose levels remained normal (90 mg/dl) until 7 weeks, and then declined to about 40% of normal at 10 weeks. A similar conclusion was reported by Bardos and Pusztai (1989) and Shankar et al. (1990).

A positive relationship similar to that between vitamin A and plasma glucose was found between vitamins E and blood glucose (Ojo et al., 1986). On the other hand, Emil and Viera (1983) reported that vitamin C deficiency can provoke glycoregulatory disorders analogous to diabetes and vice-versa. These associative disorders can be resolved by increasing vitamin C intake.

Previous results support the present study, which is in agreement with Ismail et al. (1992a), who failed to detect any significant differences in plasma glucose with 8 levels of vitamin A during a five week experimental period.

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