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Interaction of zinc and vitamin A in rats receiving a regional diet of Manaus, Amazonas, Brazil. Effect of supplementation with vitamin A, zinc and zinc and vitamin A.

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RESUMO. Interação de zinco e vitamina A em ratos recebendo uma dieta regional de Manaus, Amazonas, Brasil. Efeito da supplementação com vitamina A, zinco e zinco e vitamina A. Foi estudada a interação de zinco e vitamina A em ratos recebendo uma dieta regional de Manaus, suplementada com vitaimna A, zinco e zinco e vitamina A. A diet regional foi elaborada segundo os dados de Shrimpton & Giugliano (6), para famílias com rendimentos inferiores a 2 salários mínimos. O ensaio biológico para estudo da interação, se baseou na depleção de zinco e vitamina A em ratas na fase de lactação, e na repleção dos filhotes após o desmame,onde se realizaram as suplementações com zinco (0,82 mg %) e vitamina A (94,2 µg %) isoladamente e em conjunto, de acordo com as recomendações do Committee on Laboratory Animal Diets (7). Os resultados nos permitiram concluir que ocorreu interação destes nutrientes a nivel de mobilização de vitamina A hepática. Na dieta estudada existe necessidade de suplementação com vitamina A, para manutenção das reservas hepáticas, e, com zinco, para a manutenção normal da concentração de vitamina A no plasma, embora a quantidade de zinco presente, na dieta, avaliada pelos parâmetros de medida da biodisponibilidade de zinco como crescimento, concentração nos orgãos e da enzima zinco dependente tenha sido utilizada adequadamente pelos animais, provavelmente pelos fatores promotores da dieta.
Palavras chave: Zinco, vitamina A, interação, suplementação e dieta.

SUMMARY. The interaction of zinc and vitamin A in rats receiving a regional diet of Manaus, supplemented with vitamin A, zinc and zinc and vitamina A was studied. The regional diet was elaborated according to data of Shrimpton and Giugliano (6), for families receiving less than two minimun salaries. The biological test to study the interaction was based on the depletion of zinc and vitamin A in rats in the period of lactation, and a period of repletion where supplements of zinc (0.82 mg %) and vitamin A (94,2 µg %) were given, either separately or together, according to the recommendations of the Committee on Laboratory Animal Diets (7). From the results, it was concluded that there was an interaction of these nutrients in terms of mobilization of hepatic vittamin A. Although the regional diet of Manaus did not meet the zinc RDA, the amount present was enough to utilize the available vitamin A. Although the amount of zinc present in the diet, as determined by parameters of biovailability, such as growth, concentration in organs and zinc-dependent enzymes, was adequately used by the animals, probably due to promoting factors in the diet. The Manaus regional diet needs to be supplemented with vitamin A in order to maintain the hepatic reserves, and with zinc, to maintain the normal levels of vitamin A in plasma.
Key words: Zinc, vitamin A, interaction, supplementation and diet.

INTRODUCTION

Zinc and vitamin A are essential nutrients for human and animals, and despite the knowledge accumulated during years, information about their interaction in the organism is only recent (1). The interaction of zinc and vitamin A became evident when rats deficient in zinc, showed low levels of plasma vitamin A, despite having an adequate dietary intake of this vitamin (2). Diet deficient in zinc in an experimental model, appears to influence not only the mobilization of vitamin A, but also its oxidation/ reduction interconversion in periferal tissues (3).

Specific studies involving the diet of a population in order to verify the interaction of zinc and vitamin A are scarce in the literature. Therefore, taking into consideration the severity of vitamin A deficiency in the Amazon region (4) and the indication of zinc deficiency (5), it was decided to study the interaction of these nutrients in a diet of the Manaus region, in rats, as well as, to verify the effect of supplementation with vitamin A, zinc and zinc and vitamin A.

The utilization of this model, in rats, have been reported in the literature for studies of these nutrients, and the extrapolation for humans can be done with some precaution.

MATERIAL AND METHODS

Diet: The diets simulated the regional diet of Manaus, which was elaborated according to the data of Shrimpton and Giugliano (6), on basis of the average consumption of famiies receiving up to two minimum salaries. Food items were purchased from local shops in the City of Manaus (Amazonas), Brazil. The description and amount of these food items are given in Table 1.

The study was carried out in two phases, in the first, the depletion in zinc and vitamin A and in a second phase a repletion period.

In this experiments, thirteen adult female albino Wistar (*Rattus norvergicus*, var, albinus, Rodentia, Mammalia) rats with six male young rats each, were used. They were provided by University of São Paulo School of Pharmaceutical Sciences. These rats received diet «ad libitum» based on casein deficient in zinc and vitamin A in the lactating period, with the aim of obtaining recently weaned animals deficient in both of these nutrients, which corresponded to the

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depletion period. This diet contained the nutrients in quantities suggested by the Committee on Laboratory Animal Diets (7), with the exception of zinc and vitamin A (Table 2). The casein utilized was previously washed with 1 5 EDTA twice and followed washed ten times with deionized water in order to remove the residual zinc. The initial casein zinc level was 21 µg Zn/g and we obtained a final level of 4,98 µg/g.

TABLE 1
Composition of a regional diet of Manaus, AM (6)

Items	Amount (g)
Rice, raw and refined	64,7
Bread roll	140,6
Manioc, flour	71,9
Potatoes sweet/English	13,3
Sugar refined	60,4
Beans, raw	29,7
Tomato	11,5
Onion, raw	8,9
Com, boiled, cooked	7,1
Pumpkin, raw	3,5
Cabbage	2,7
Parsley	4,7
Banana	65,2
Avocado	5,8
Lemon	4,7
Beef and chicken	74,4
Eggs, raw	12,5
Fish	150,6
Milk, reconstituted	101,2
Oil, soya	16,0
Margarine	6,1
Soft drink	59,9
Coffee	12,4
Salt, refined	9,8
Miscellaneous	5,1
Total	943,0

TABLE 2
Ingredients used for preparing casein-based diets[1]

Nutrients	Control	Depleted
Casein[2]	20,0	20,0
Sugar	10,0	10,0
Fibre	5,0	5,0
Oil, soya	5,0	5,0
AIN Mineral with zinc	3,5	-
AIN Mineral mix without zinc	-	3,5
AIN Vitamin mix	1,0	-
AIN Vitamin mix without vitamin A	-	1,0
D,L - Methionine	0,3	0,3
Choline bitartrate	0,2	0,2
Starch	55,0	55,0

(1) Diets prepared according to the Committee on Laboratory Animal Diets (7)
(2) Casein washed with 1 % EDTA twice and deionized water 10 times.

At he same time, two female rats in the same conditions as above, with six pups each, were fed with a complete casein diet, and were considered the control group in order to confirm depletion. At the end of 25 days, six animals recently weaned from the control group and six from the depleted group were sacrificed to assess the nutritional status of zinc and vit. A.

After this period, the animals which were depleted in zinc and vitamin A received a regional diet of Manaus for a period of seven days, for adaptation with this diet. At the end of this period, six more animals of each group were sacrified in order to determine the parameters related to zinc and vitamin A in this time, considered the start of repletion period.

The repletion period was 30 days and the design was completely random with five groups of eight animals each, weighing 48,90±0,54 g, distributed as follows: 1) Regional Diet (RD), the animals received the regional diet of Manaus, with a zinc concentration of 2,2 mg % and vitamin A 25,8 µg %; 2) Regional Diet plus vitamin A (RD+A), the regional diet was supplemented with 94,2 µg % of retinol; 3) Regional Diet plus zinc (RD+Zn), the regional diet was supplemented with 0,82 mg % zinc; 4) Regional Diet plus Zinc plus vitamin A (RD + Zn+ A), the regional diet was supplemented with 0,82 mg % and 94,2 µg %, respectively, of zinc and vitamin A; 5) Control, received a complete diet based on casein, according to the recommendation of the Committee on Laboratory Animal Diets (7) which includes 120 µg % vitamin a and 3,0 mg% of zinc.

During the experimental period, the animals were kept in individual stainless steel cages, in an environment with controlled and constant humidity and temperature, with a light cycle of 12 hours. All material used was demineralized in 30 % nitric acid for a minimum of 12 hours.

The growth of the animals was followed during the entire study by weighing them each week. The provision of food, the change of water drinking and the collection of faces was done every two days and at the same time. At the end of the experiment, all animals were anesthetized in order to obtain the biological material for analysis. The food was restricted only on the day the animals were sacrificed, they were given deionized water «ad libitum».

Blood was taken from the abdominal artery and centrifuged at 3000 rpm for 15 min. at 4 °C. The plasma obtained was kept at 20 °C until analysis. After blood was collected, the liver was injected with freezing 0,9 % NaCl, wrapped in aluminium foil and frozen in liquid nitrogen until analysis. Testicles was wrapped in aluminium a tissue aliquot was homogenised and centrifuged at 37000 rpm for 60 min. at 4 °C in order to draw off the cytosol content of the cell for the determination of alcohol dehydrogenase activity, by the method of Bergmeyer (8). The concentration of protein in the supernatant was determined according to Lowry et al (9), using the Folin reagent, taking bovine albumin as control.

The analysis of the diets and the energy calculation was done following the AOAC methods (10). The determination of the fibre fraction was done according to the method of Asp et al (11) and the phytate by colorimetry, according to the method of Thompson and Erdman (12), modified method of Lajolo et al (13).

Zinc in the diets and femurs was determined by atomic absorption spectrophotometry method (14), by direct absorption of the samples in liquid form after a humid digestion (14). For analysis control, the recommendations of Cornelis (15) were followed, using as reference material «Total Diet 1548» of the NBS (National Bureau of Stantdards). The zinc concentration in plasma was determined directly in samples diluted in the ratio of 1:4 by the atomic absorption method according to Rodrigues et al (16).

The concentrations of calcium and iron in the regional diet was determined by plasma induced atomic emission spectroscopy and selenium by neutron activation analysis.

The concentrations of vitamin A and carotenoids in plasma and liver of the animals was determined by the method of Bessey, Lowry et al (17) modified by Flores (18).

Analysis of variance and Tuckey test were used at a probability of 5 % in order to compare the means, Pimentel Gomes (19).

RESULTS AND DISCUSSION

Diet: Evaluating the quantitative aspect and the extrinsic or dietetic factors, it can be seen that the regional diet of Manaus presents relevant aspects which favour the bioavailability of zinc, such as, high protein content (96.8 g/day) of mainly animal origin, deficiency of calcium (405.5 mg), low level of phytate (403 mg), phytate: zinc molar ratio (3,8) and phytate x Ca/Zn (38,7) below the critical levels, being adequate in fibre (19.6 g), with bordeline levels of zinc (10.7 mg), and deficeincy of vitamin A (126.4 µg/day). Therefore, it is possible that all these predisposing factors have influenced psoitively in the results obtained, despite the limitations of some nutrients.

Depletion period: Signs of deficiency were observed in the young rats which suckled zinc and vit A depleted mother. The animals showed weight loss, reduced appetite, loss of fur and abnormal posoture.

Analysis of Table 3 indicates that there was a significant difference for ponderal growth.

TABLE 3
Average values and standard deviations of weight gain, zinc levels in the femurs and vitamin A concentration in plasma and liver of animals in the depleted and control groups during the period of development of zinc and vitamin A deficiency

Group	Weight variation (g)	Concentration Vitamina A		Zinc
		Plasma (%)	Liver (µg/g)	Femurs (µg/g)
Deplete	$49{,}9^{b} \pm 0{,}5$	$8{,}9^{b} \pm 3{,}4$	$26{,}4 \pm 5{,}8$	$35{,}5^{b} \pm 3{,}3$
Control	$103{,}2^{a} \pm 3{,}9$	$31{,}5^{a} \pm 2{,}5$	$22{,}6^{a} \pm 2{,}1$	$115{,}7^{a} \pm 5{,}4$

The same letters in a vertical column are not significantly different at a probability level of p<0,05.

The vitamin A concentration in plasma, and zinc concentration in femurs, were significantly different for the depleted and control groups (p<0,05), demonstrating that depletion had occurred. With regard to vitamin A concentration in liver, there was no significant difference between the depleted and control groups (p<0,05) (Table 3). This suggests that in zinc and vitamin A deficient animals, the vitamin A was not sufficiently mobilized from the liver to plasma (Table 4). These data agree with those reported by Smith et al (2).

Repletion period: In Table 4 the food consumption and weight variation of the animals during the repletion period is presented. It was noted that during the experimental period, all animals receiving different treatments gained weight. However, it was greater for the animals treated with the regional diet of Manaus, whether supplemented or not.

TABLE 4
Average values and standard deviation of food consumption and weight gain

Group	Food consumption (g)	Weight variation (g)
RD[1]	$388{,}3^{a} \pm 28{,}7$	$135{,}6^{a} \pm 12{,}7$
RD + A	$390{,}5^{a} \pm 43{,}4$	$144{,}2^{a} \pm 15{,}9$
RD + Zn	$346{,}0^{ab} \pm 34{,}2$	$125{,}9^{ab} \pm 15{,}9$
RD + Zn + A	$376{,}1^{a} \pm 41{,}4$	$141{,}9^{a} \pm 16{,}6$
CONTROL	$303{,}3^{a} \pm 33{,}5$	$99{,}3^{a} \pm 16{,}0$

1 RD (Regional diet); A (vitamin A); Zn (zinc)
The same letters in a vertical columm are not significantly different at a probability level of p<0,05.

On comparing the diet consumption between the groups, it can be seen that the control group consumed the least and the control diet contained levels of zinc and other nutrients which met the Committee on Laboratory Animal Diets recommendations (Table 4). A possible explanation for the lower consumption of this group could be due to the fact that the casein used in the preparation of the diet was washed with EDTA in order to make the zinc levels uniform, and this process could have affected the palatability and acceptability of this diet, as shown by Cossack and Hamer (20).

Zinc levels in femurs were greater in those animals whose diet was supplemented with zinc, vitamin A and zinc and vitamin A. Table 5.

TABLE 5
Mean and standard deviation of zinc level in femurs and plasma of animals subjected to different treatments during the repletion period

Treatment	Zinc Femurs (µg/g)	Plasma (µg %)
RD	111.7 ± 7.7	1.5 ± 0.1
RD + A	116.0 ± 5.8	1.6 ± 0.1
RD + Zn	120.3 ± 7.0	1.5 ± 0.1
RD + Zn + A	119.5 ± 11.0	1.7 ± 0.1
Control	98.8 ± 15.2	0.8 ± 0.2

The same letters followed in a vertical column are not significantly different at a probability level of p<0,05.

These results suggested that the zinc in the regional diet of Manaus is available, and the deficiency in calcium could increase the retention of zinc in the femurs (21).

The zinc plasma values were greater when the regional diet was supplemented with zinc and vitamin A, and in decreasing order for those on the diet supplemented with vitamin A, compared with the control animals (Table 5). Second King (22) the alterations in zinc plasma concentration are temporary, tending to reach a homeostatic equilibrium, and are greatly influenced by a variety of factors, such as physical stress, infection, inflamation, hormonal conditions, amongst others (23).

As far as the alcohol dehydrogenase (ADH) activity in the animal liver is concerned, it was observed that there was a greater activity, $p<0.05$, when the animals were treated with the regional diet supplemented with zinc (Table 6), but not when was accompanied by additional vitamin A.

Boron et al (23) showed an alcohol dehydrogenasse activity significantly reduced inzinc deficient animals. Other studies have shown conflicting results (3).

TABLE 6
Mean alcohol dehydrogenase (ADH) activity in liver and testicles of animals submitted to the different treatments in the repletion period

Treatments	ADH activity (nmol/min/mg protein)	
	Liver	Testicles
RD	$51.4^{b} \pm 13.3$	$23.7^{a} \pm 2.6$
RD + A	$61.0^{b} + 11.4$	$25.5^{a} \pm 5.6$
RD + Zn	$78.8^{a} \pm 10.9$	$21.1^{a} \pm 6.7$
RD + Zn + A	52.8^{b} 12.8	$27.3^{a} \pm 8.0$
Control	$57.4^{b} \pm 13.6$	$21.3^{a} \pm 3.6$

The same letters appearing vertically are not singifantly different at a probability level of $p<0.05$

In relation to the activity of this enzyme in testicles of the animals in the different treatment groups, there was no significant difference $p<0.05$ (Table 6).

The plasma vitamin A levels were significantly greater in the animals whose diet was supplemented with zinc and vitamin A, compared with other treatment (Table 7).

According to studies of Underwood et al (24), the concentration of vitamin A in plasma is directly related to the concentration of vitamin A in liver, only when the hepatic stock is below the threshold.

TABLE 7
Means and standard deviation of vitamin A concentration in plasma and liver of animals submitted to different treatments during the repletion period

Treatments	Plasma vit. A (µg %)	Liver vit. A (µg/g)
RD	$26.1^{b} \pm 5.4$	$5.5^{d} \pm 1.1$
RD + A	$22.7^{b} \pm 5.4$	$42.0^{a} \pm 4.3$
RD + Zn	$22.6^{b} \pm 4.8$	$7.7^{cd} \pm 1.4$
RD + Zn + A	$33.2^{a} \pm 4.8$	$31.0^{b} \pm 5.8$
Control	$22.4^{b} \pm 3.1$	$14.2^{c} \pm 3.0$

The same letters appearing vertically are not significantly different at a probability of $p<0.05$.

A number of dietetic conditions other than vitamin A defciency can reduce the plasma vitamin A levels. For example, rats receiving a protein deficient diet (25) or a limited complete diet (2). Another possibility is that the zinc present in the diet was sufficient to maintain the plasma levels by means of its specific effect on the mobilization of hepatic vitamin A, according to studies of Smith et al (2).

The hepatic levels of vitamin A were significantly greater in the animals whose vitamin A, followed by those supplemented with zinc and vitamin A (Table 7), which suggest that vitamin A is the limiting nutrient in the diiet. Such evidence can be justified by the low amounts obtained in the chemical analyses, and by the hepatic concentration, which was significantly reduced in the animals which received a regional diet and a regional diet supplemented with zinc.

It was also observed that the regional diet supplemented with both zinc and vitamin A appears to have influenced the hepatic reserves as well as a greater mobilization of vitamin A from the liver, which is justified by the increase in plasma levels.

These results allows the interpretation that zinc dietary levels, according to the recommendations of Committee on Laboratory animal Diets (7), are important for a normal vitamin A metabolism and that vitamin A is important in maintaining normal hepatic levels.

Therefore, it can be concluded that in the conditions of this study, there was an interaction of the nutrients studied, by the effect of zinc on the mobilization of vitmain A in the liver.

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