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WEIGHT GRADIENT AND PHYSIOLOGICAL RESPONSES TO CATION-TREATMENT BY SALMONELLA ENTERICA- INFECTED RABBITS

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Abstract

Interest in immunomodulators is increasing following the recognition that positive immunomodulators could be useful intervention tools in the control of diseases and infections. An attempt to determine the effects of some of the cations on body weight and physiological reactions was carried out. Thirty five female adult New Zealand white rabbits grouped into seven, 5 pairs per each of these cations (Zn²⁺, Cu²⁺ and Mg²⁺), and supplemented with 1ml/day of single and double strength concentrations of cation for 24 days; the control was not supplemented with any cation. During the study period the rabbits were fed with Guinea grower mash and water *ad libitum*. There was regular taking of body weight of the rabbits using a top-loading weighing balance, while feed consumption, rectal temperature, stool frequency, physical appearance and behavioural changes were noted. Weight gradient studies show gradual increase in body weight following cation treatment of rabbits, but after challenging the various groups with oral administration of 0.5 ml of 10⁶ CFU / ml of saline suspension of *Salmonella enterica* for three exposures on alternative days, there was a progressive decrease in body weight of rabbits. However, there was no significant difference in stool dropping, body weakness, dullness and rough furs in all the groups. Effects were more pronounced in control group than in cation supplemented groups. This result, therefore, provide evidence of the significance of zinc, copper and magnesium oral supplementation in mammals and, of course among these three cations, copper appears to be more effective in improving body weight gain, though the mechanism is not known.

Keyword: Trace elements, Body weight, Physiological responses, *Salmonella enterica*

INTRODUCTION

The significance of the biochemical and nutritional roles of trace elements is widely recognized, since trace elements are found as constituent components of many metalloproteins and metalloenzymes. Trace elements are substances (elements) found in trace amounts in food. They are essential for the normal functioning of the body system though in trace amounts. The definitive feature of a nutritionally significant trace element is either its essential interaction in physiological processes or its potential toxicity when present at above optimal concentration in tissues, food or drinking water. They are also important components of hormones and certain factors with special physiological functions (1). Arbitrarily, the term 'trace' has been applied to concentrations of elements not exceeding 250 g per gram of matrix (2).

An element is considered essential to an organism when reduction of its exposure below a certain limit results consistently in a reduction in physiologically important function or when the element is an integral part of an organic structure performing a vital function in that organism. However, not all essential elements are of equal practical importance for public health. Under-exposure, over-exposure or both is known to occur under certain environmental condition for selenium and zinc, whereas, fluorine and the heavy metals cadmium, mercury and lead are of great concern because of the concentration occurring in food chains (3).

At the tissue level, domestic animals require essentially the same amount of minerals per comparable unit of function performed. This does not imply, however, that the dietary intake should be equal. On the contrary, differences in the percentage concentration of the diet should be expected. Factors affecting dietary mineral requirement include the

amount of food ingested per unit of weight, growth rate and species of the mammal, ambient temperature, kind of feed

supplied and dietary balance of other nutrients, and level of antagonism in the ratio and age of the animal. It is evident that younger animals absorb higher and faster than the older ones.

Cations are immunomodulators from miscellaneous sources. Immunomodulators are biological response modifiers which specifically interfere in cellular and humoral immune mechanisms and are also involved in many biochemical processes supporting life such as metabolism and sequestration of free radicals. The relation between trace elements and human health has been noted (4). With respect to cardiovascular diseases and hypertension, attention has mostly been focused on manganese, zinc, arsenic, cadmium, lead, selenium, vanadium, copper, cobalt, chromium and fluorine (5). But the effects of cations on gross weight of individual remain unknown. Hence, the research on the physiological responses to cation treatment was designed with an aim to examine physiological reactions and changes in body weight, as well as pathological changes and specific organ weight which are not reported in this paper.

MATERIALS AND METHODS

Laboratory animals-

The experimental animals comprised of 35 female adult New Zealand white rabbits, obtained from the animal house of the Department of Microbiology, University of Benin, Benin City.

Organic salt - The salts (copper sulphate, zinc sulphate and magnesium sulphate) were obtained from the laboratory of the Department of Microbiology, University of Benin, Benin City. A doubling concentration of each cation was employed

in treatment of the rabbits, so that the effects observed if due to treatment, should also show multiplying effects. The treatment dosages were also chosen in relation to values obtained from human population studies carried out elsewhere (3). Treatment groups and supplementation given were group 1 (Mg x 1) 10.0 µg / ml, group 2 (Mg x 2) 20.0 µg / ml, group 3 (Zn x 1) 16.0 µg / ml, group 4 (Zn x 2) 32.0 µg / ml, group 5 (Cu x 1) 2.0 µg / ml, group 6 (Cu x 2) 4.0 µg / ml, and the control group which was not supplemented with any cation. The treatment groups were separately given oral supplements of zinc, magnesium and copper respectively for 24 days before being challenged with oral administration of saline suspension of pure culture of *Salmonella enterica* with a hypodermic syringe bearing 0.5 ml of 10⁶ CFU / ml of the organism for three exposures on alternate days. During the entire study period, the rabbits were fed Guinea grower mash (Bendel Feed and Flour Mills, Ewu, Edo State) and water from University of Benin borehole, Benin City. Stool frequency was specifically noted and the body weight was taken regularly using a top-loading weighing balance (Five Goats, China) while their physical appearance, feed consumption, rectal temperature and behaviours were recorded daily.

RESULTS

Weight gradient - The physiological responses of the rabbit's treatments with cation and subsequent challenge with *Salmonella enterica* are shown in table 1 below. There was an initial increase in body weight of rabbits from a mean 1.70 kg to 2.00 kg for Mg x1, 2.10 kg to 2.45 kg for Mg x 2, 2.10 kg to 2.25 kg for Zn x 1, 1.80 kg to 2.00 kg for Zn x 2, 1.80 kg to 2.30 kg for Cu x 1, 1.70 kg to 2.05 kg for Cu x 2 for the treatment

group and 1.85 kg to 2.00 kg for the control group. There was a significant increase in body weight following cation treatment of the rabbits, but after challenging the various groups with *Salmonella enterica*, there was a progressive decrease in body weight of rabbits from a mean 2.00 kg to 1.90 kg for Mg x1, 2.45 kg to 2.30 kg for Mg x 2, 2.20 kg to 2.00 kg for Zn x 1, 2.00 kg to 1.80 kg for Zn x 2, 2.30 kg to 2.23 kg for Cu x 1, 2.05 kg to 1.85 kg for Cu x 2 for the treatment groups and 2.00 kg to 1.75 kg for the control group. There was no statistically significant difference in the net weight loss in all the groups and the control at P < 0.05. Other observed physiological effects of exposure to the challenge were body weakness, dullness, rough fur and loss of appetite. These effects were more pronounced in the control rabbits than in the cation - supplemented groups. Despite the possible effects of toxicity on the groups treated with double strength of the cations, they however, survived the experimental period. There was no significant frequency in stool in the test period before and after challenge with *Salmonella enterica*. Seven days after challenge there were obvious signs of infection of the rabbits: body weakness, lack of appetite, generally rough furs in all the groups. The physiological effects were more on the control group than any other group.

Physiological signs and clinical scores

There was no difference in the rectal temperature before and after the challenge with *Salmonella enteric*. The physiological changes observed were scored positive: mild reactions as (+), Light reactions as (++) , and heavy reaction as (+++) or more according to the degree of infection.

TABLE I: MEAN WEIGHTS (KG) OF RABBITS SUPPLEMENTED WITH CATIONS AND CONTROL

Period	GROUPS						
	Mg x1	Mg x 2	Zn x 1	Zn x 2	Cu x 1	Cu x 2	Control
Initial	1.70	2.10	2.10	1.80	1.80	1.70	1.85
1 st week	1.78	2.26	2.16	1.90	2.01	1.78	1.82
2 nd week	1.88	2.32	2.20	1.94	2.14	1.81	1.85
3 rd week	2.00	2.45	2.25	2.00	2.30	2.05	2.00
4 th week	1.90	2.35	2.00	1.80	2.23	1.85	1.75
Mean ±SE	1.85 ±0.1	2.30 ±0.1	2.14 ±0.1	1.89 ±0.0	2.10 ±0.1	1.84 ±0.1	1.85 ±0.1

All values are means ± SEM of 5 values per group for each supplementation

TABLE II: PHYSIOLOGICAL CHANGES FOLLOWING *SALMONELLA ENTERICA* CHALLENGE

Parameters	GROUPS						
	Mg x 1	Mg x 2	Zn x 1	Zn x 2	Cu x 1	Cu x 2	Control
Rectal temperature	N	N	N	N	N	N	N
Body weakness	+	++	+	++	+	+	+++
Lack of appetite	++	++	++	++	++	++	++
Rough furs	+	++	+	++	+	+	+++
Stool droppings	N	N	N	N	N	N	N
Weight (Kg) gained before challenge	0.30	0.35	0.15	0.20	0.50	0.35	0.15
Weight (Kg) loss after challenge	0.10	0.10	0.25	0.20	0.70	0.20	0.25

Key: + = mild signs, ++ = light signs, +++ = heavy signs, N= normal range 36 - 37°C

DISCUSSION

Attempt was made in this study to determine the physiological responses of rabbits to treatments with trace elements, the effects of the cations on body weight and their physiological reactions to the cations. It was observed that exposure to cations as oral supplements enhanced body weight gain. The body weight gained was concentration -

dependent among those treated with zinc and magnesium, though not statistically significant at P < 0.05. The reverse was the case among those treated with copper, where there

was an appreciable increase in body weight of those treated with 2.0 µg of copper, than those of 4.0 µg, and the difference was not concentration -dependent. It is therefore implied that cations improved metabolic activities in the affected rabbits, an observation which is not at variance with the report of Camara and Amaro (6) who in their various studies indicated the importance of cations in mammalian metabolism.

Rabbits on cation supplement showed higher resistance to the damaging effects of *Salmonella enterica* as evidenced by the significant difference in the physiological responses of the treatment group of rabbits and the control. This finding may be related to the improved nutrition, which may have impacted on the immunity of the cation - supplemented groups. The relationship between nutritional status and immune functions has been reported by several authors (7, 8, 9 and 10). The result of the present study, therefore, provides evidence of the significance of zinc, copper and magnesium oral supplementation in mammals and of course among these three cations, copper appears to be most effective in improving body weight gain and physiological responses to the overwhelming effects of *Salmonella enterica*, though the mechanism is yet unknown. The potential effects of trace elements cannot be over emphasized during treatment of

infections and convalescence. Nutritional supplements rich in essential trace elements should also be considered for recovery of weight loss due to infection, to improve metabolism and body activities. There is therefore, the need for further studies to explore the potential of trace elements on body weight in health and specifically as a tool to combat uncharacterized weight gain or loss in our society especially among children of school age and institutionalized adults living in the sub-sahara of Africa. We recommend that urgent action be taken by government at all levels to acquire more information not only on staple foods of developing countries but also to determine the effect of cultural, climate and environmental changes on the contents of these elements in diets derived from such foods.

Conclusion:

The physiological changes during the assessment indicate that the study cations impacted on the affected rabbits and improved metabolic activities which brought about progressive weight gain *ab nisu*. Consequently, higher resistance to the damaging effect of the *S. enterica* challenge was evident in physical parameters observed in the treatment group and not in the control group.

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