

# **EFFECTS OF CATION TREATMENT ON BODY - ORGAN WEIGHT RATIO AND PATHOPHYSIOLOGICAL CHANGES IN PERITONEUM CAVITY OF FEMALE NEW ZEALAND RABBITS**

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## **Abstract**

The role of trace elements in normal function of the body cannot be over emphasized. This study was carried out to determine the effects of oral administration of zinc ( $Zn^{2+}$ ), copper ( $Cu^{2+}$ ) and magnesium ( $Mg^{2+}$ ) on body, organ weight and pathophysiological changes in the peritoneum of New Zealand White rabbits. Standard solutions of the cationic salts were prepared using sterile distilled water to obtain two concentration of each cation. The rabbits were distributed into seven groups: Group I was administered 10.00  $\mu g / ml$  of  $Mg^{2+}$ , Group II received 20.00  $\mu g / ml$  of  $Mg^{2+}$ , Group III received 16.00  $\mu g / ml$  of  $Zn^{2+}$ , Group IV received 32.00  $\mu g / ml$  of  $Zn^{2+}$ , Group V received 2.00  $\mu g / ml$  of  $Cu^{2+}$ , Group VI received 4.00  $\mu g / ml$  of  $Cu^{2+}$  while, group VII were left untreated with any cation supplement. The increase in body, and organ weights in the rabbits treated the cations was concentration dependent in  $Zn^{2+}$  supplemented group ( $P > 0.05$ ), but not in  $Cu^{2+}$  and  $Mg^{2+}$  groups. The increment in mean body – organ ratio of treated rabbits was statistically significant ( $P < 0.05$ ) when compared with untreated rabbits.  $Mg^{2+}$  showed more fat deposit followed by  $Cu^{2+}$  groups but none in  $Zn^{2+}$  groups. It was therefore, concluded that oral supplements of copper, zinc and magnesium caused changes in body weight and weight of organs of spleen, liver, lungs, kidneys and ovaries. The excess fat deposit around some of the internal organs may impede its normal physiological function.

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**Keywords:** Body weight, Organ weight, Cation treatment, Fat deposit, New-Zeeland rabbits

## **Introduction**

The role of micro mineral in health cannot be over emphasized, zinc been a modifier of wide spectrum of biological activities and its deficiency has been related to various dysfunctions and alterations of normal cell metabolism (Alikwe *et al.*, 2012). Of 95 naturally occurring elements of periodic table, about 25 are essentially needful for the normal functioning of the body, and are required in small amount (Nelson, 1997). They are usually found in small amount in foods. The nutritional significance of trace elements have been documented, describing its' essential interaction in physiological process or it potential toxicity when present at a level higher than optimal concentration in tissue, food or drinking water (Park, 2007; Ojiezeh *et al.*, 2012).

The dietary deficiency of these elements associated with variety of clinical features has been documented. Their functions have also been said to be important component of hormones and special physiological process including catalytic, structural and regulatory activities in which they interact with macromolecules such as enzymes, pro-hormones, pre-secretory granules and biological membranes (Aggett, 1985; Zlotkin *et al.*, 1995). In a study, zinc, magnesium and copper were reported to have improved activities that brought about progressive weight gain, and higher resistance to damaging effects on tissues in *Salmonella enterica* challenged rabbits (Ojiezeh *et al.*, 2012; Ojiezeh, 2013).

The adverse effects of maternal malnutrition have been well documented – maternal depletion, low birth weight, anaemia, pregnancy, toxaeimias, post- partum haemorrhage, all leading to high mortality and morbidity. The effects of malnutrition are also frequently more serious during the formative years of life (Park, 2007). The reviewed work of Zlotkin *et al.* on serum biochemistry of some of the elements in preterm described clinical deficiency states and provided rational for recommended enteral and parenteral intake for preterm infants (Zlotkin *et al.*, 1995). With background knowledge of the effects of good dietary food, enriched with micro and macromolecules, it is possible to develop appropriate tools for intervention in the cycle of human diseases and reduce mortality. The present study focuses on the interaction of cations on the body and organ(s) weights and fat deposit around the organs. It is hoped that the findings will enrich knowledge on the effects of trace element in public health.

## **Materials and methods**

### **Laboratory animals- rabbits**

Thirty five female New Zealand white rabbits used for this research were weighing between 1.70 - 2.10 kg and were obtained from Animal house, University of Benin, Benin City.

### **Organic salts**

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.

### **Treatment of rabbits**

The rabbits were assigned using a complete randomized design, to seven different treatment groups of 5 rabbits per treatment and were housed in different nest cages of wire mesh with dimension of 1.2 m by 0.6 m per rabbit. The cages were open sided for cross ventilations. Quarantine was for 30 days and there were no observable disease symptoms before the commencement of the experiment. The treatment dosage was chosen in relation to values obtained from studies carried out elsewhere (Ojiezeh *et al.*, 2012). Treatment groups and supplementation concentration given were: Group 1 (Mg x 1) 10.0 µl / ml, group 2 (Mg x 2) 20.0 µl / ml, group 3 (Zn x 1) 16.0 µl / ml, group 4 (Zn x 2) 32.0 µl / ml, group 5 (Cu x 1) 2.0 0 µl / ml, group 6 (Cu x 2) 4.0 µl / ml and the control group was not supplemented with any cation. The treatment groups were separately given oral supplements of zinc, magnesium and copper respectively for 24 days. The physiological responses (though not reported in this paper) were observed before sacrificing on the 30<sup>th</sup> day. Maintenance and treatment of rabbits were in accordance with the principles of the “Guide for care and use of laboratory animals in research and teaching” prepared by the National Academy of Sciences and published by the National Institute of Health (NIH) publication 86 – 23 revised in 1985 (NRC 1985). As approved by the ethical and research committee, Achievers University, Owo. During the entire study the rabbits were fed with Guinea grower mash (Bendel Feed and Flour Mills, Ewu, Edo State) and water from University 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 (though reported in this paper).

## Harvesting of organs

A whole rabbits was weighed before sacrifice. It was anesthesia with chloroform soaked in cotton wool in a glass hood; the limbs were clipped on flat wood and ventral dorsal opened with a pair of scissors and sterile surgical blade. The organs were harvested and weighed immediately on electronic balance (MP 10001, USA) and the fat deposits and other pathophysiological changes around the peritoneum (internal organs) were noted.

## Statistical analysis

Data were expressed as mean  $\pm$  SEM. Statistical analysis was performed using ANOVA. Inter-group comparism was achieved by Duncan Multiple Range Test using SPSS 11.0. Differences were judged to be statistically significant when p was less the 0.05.

## Results

The effects of Zn<sup>2+</sup>, Cu<sup>2+</sup>, and Mg<sup>2+</sup> supplements on body and organs weights of rabbits is shown on Table 1. There was an increase in mean body weight and organs weight ratio of the supplemented groups when compared with the control group that recorded 20.70, the difference was statistically significant (P < 0.05). The increment was concentration dependent in zinc supplemented group (statistically not significant at P > 0.05), but not in magnesium and copper supplemented groups. The increase in weight of spleen, kidney and ovary of group supplemented with Mg<sup>2+</sup> may be attributed to the mean body weight of the group and a direct effect of the supplement.

Table 1: Effects of Zinc, Copper and Magnesium treatment on body and organs weights of rabbits

	Mean body weight (g)	Total organ weight (g)	Body weight - organ ratio	Spleen (g)	Lungs (g)	Kidneys (g)	Liver (g)	Ovary (g)
Mg1	1850 $\pm$ 0.1	57.32	32.28	0.86	7.69	10.14	39.10	0.53
Mg2	2300 $\pm$ 0.2	80.87	28.44	1.01	8.98	13.08	56.81	1.01
Zn 1	2140 $\pm$ 0.2	72.27	29.61	0.72	9.69	11.14	52.19	0.54
Zn2	1890 $\pm$ 0.1	61.93	30.52	0.72	9.52	10.24	40.87	0.58
Cu1	2100 $\pm$ 0.2	68.65	30.59	0.67	8.51	10.14	49.00	0.43
Cu2	1840 $\pm$ 0.1	64.14	28.69	0.87	10.03	11.06	41.48	0.70
Con trol	1840 $\pm$ 0.3	80.89	20.70	0.44	11.20	11.54	57.29	0.42

Significant difference at P < 0.05

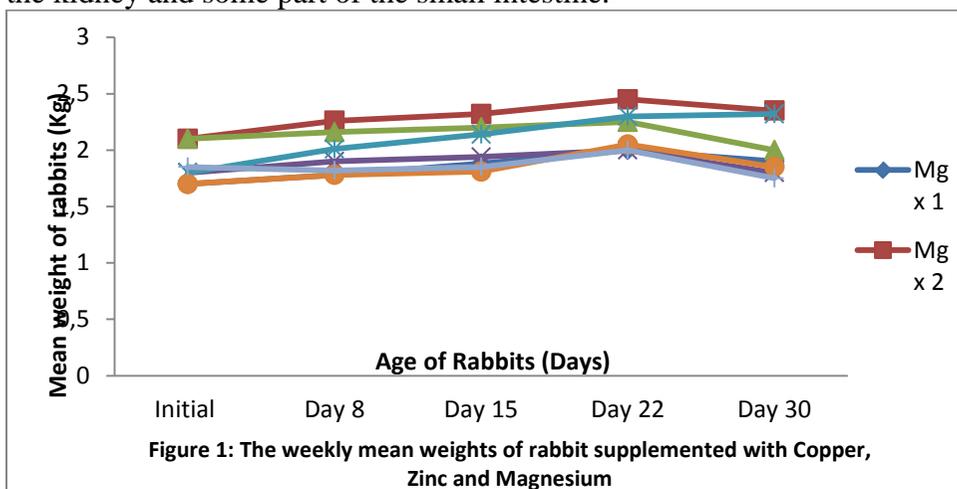
All values are means  $\pm$  SEM of 5 values per group for each supplementation

Table 2: Pathophysiological changes on the peritoneum of rabbits supplemented with zinc, copper and magnesium

Organ Structure	Mg x 1	Mg x 2	Zn x 1	Zn x 2	Cu x 1	Cu x 2	Control
Rectal temp	N	N	N	N	N	N	N
Tenderness of liver and lungs	+	++	N	N	N	N	N
Ovary deformation	+	++	N	N	N	N	N
Fat deposit around the GIT	+++	+++	N	N	+	+	N
Nodules around the kidneys & GIT	N	N	+	+	N	N	N

*GIT= Gastrointestinal tract*  
 +++ = Heavy, ++ = Moderate, + = light, N = normal

The pathophysiological changes observed on the peritoneum (internal organs) of rabbits supplemented with Zinc, Copper and Magnesium are shown on Table 2. The results showed heavy fat deposit on the wall of the gastrointestinal tract (GIT), Ovary deformation and tenderness of organs of lungs and liver as a result of the effects of oral supplement of Mg<sup>2+</sup>. Fat deposits (light) were also seen around GIT, the ovary and the kidneys of rabbits supplemented with single and double strength of Cu<sup>2+</sup>. There was no fatty deposit in rabbits supplemented with zinc but some nodular swollen on the kidney and some part of the small intestine.



The weekly mean weights of rabbit supplemented with copper, zinc and magnesium are shown in Figure 1. There was a progressive increase in

weight gain in all the groups until the incidental inoculation of the Salmonella. The effects of salmonellosis were seen as loss of appetite, rough fur and loss of body weight (Though not reported in this paper).

## Discussion

The administration of Zn, Mg and Cu to normal rabbits produced no toxic effect on the tissues. This is revealed by non-significant difference in the body-organs ratio weights of the liver, lungs, kidneys and spleen in the supplemented group compared to normal control group. The study showed a progressive weight gain in the rabbits in the first three weeks of the study, which is analogous with normal development of animals. This means that the treatment had no negative impact on the wellbeing of the rabbits. This observation is in line with Ibrahim and Mat (1995), who's results showed linear relationship between total metal concentration and body weight in marine bivalve *Anadara granosa*, and agree with the report of Ojiezeh *et al.* (2012) on weight gradient and physiological responses of rabbit's growth and development on treatment with cations.

This increase in body weight may be associated to the various concentrations of the trace elements used in this study. Since magnesium is required for the proper growth and maintenance of bones and also required for the proper function of nerves, muscles, and many other parts of the body (Guyton and Hall, 2006). The magnesium used in this study may have enhanced proper growth of bones and other body tissues leading to the increase in body weight. In addition, zinc had earlier been reported to improved linear growth and weight gain (Pochon, 1981; Zemel *et al.*, 2002; Brzozowska *et al.*, 2006; Ojiezeh *et al.*, 2012) and improved the metabolic activities and enhanced litter sizes in rabbits treated with 20µg and 40µg as reported by Alikwe *et al.* (2012), an observation that is in agreement with the report of Hurley and Swenerton (1966), who in an earlier study observed a reduced litter size in zinc deficient sows. It has been documented that Copper treatment caused a significant high body weight gain (Turin *et al.*, 1997) as was observed in this study. The reduction in weight observed at the fourth week was due to physiological adjustment to the incidental bacteria challenge.

Though the Body - organs weight ratio of zinc supplemented rabbits was concentration which suggest that increase in Zn intake may essentially have no multiplier effect on the organ weights, but, perhaps improved specific function of the organs especially organs of the lungs and ovaries as revealed in this study. This finding is in tendon with the report of Alikwe *et al.* (2012) who documented an increase in performance of rabbits supplemented with zinc of 20 µg and 40 µg for 60 days. However, the concentrations of Zn, Mg and Cu used in this study impacted the weights of

organs of the spleen, ovaries and kidneys but not the Lungs and liver. The reduction in weight of liver and lungs of the treated groups may be due to intoxication. Perhaps, the exposure was becoming prolonged. And the liver been a processing station where metabolites are detoxified, may be actively involved in regulating the body homeostasis.

The pathophysiological changes observed in the treated rabbit's calls for caution in the use of cations as supplements in patients. The benefits of the trace elements cannot be over emphasis but the adverse effects should be weighed as well. The deposit of fat around the GIT of rabbits supplemented with Mg and Cu: the heavy fat deposit may hamper the normal functioning of the organ and impulse system. The tenderness (soft texture) of liver and lungs of the affected rabbits could lead to distorted functioning of the organs. Traditionally, adipose tissue was seen only as a depot for energy storage. However, recent discoveries on the physiological effects of specific fatty acids and their isomers indicate lipid may be involved in a much more intricate molecular regulatory system than was originally recognized (Pariza *et al.*, 2000; Arab, 2003).

Light fat deposits were also observed around the gastrointestinal tract as a result of the single and double concentration of Cu supplementation which means that intake of Cu would enhance fat deposition in the body. This finding is in tandem with the report of Elliot and Bowland (1970) and Huang (2013) who in separate reports found heavy fat deposition in pork and deposit of fat in goat meat respectively following oral supplement of Cu. Similarly, Solaiman *et al*, (2006) had explained the role of Cu in fat deposition, serum lipid profile, carcass traits and carcass composition of goat kids'.

The nodular swollen around the kidneys of rabbits and GIT in zinc supplemented group may be as a result of immunologic reactions to the bacterial challenge. Zn had been earlier reported to enhance immunological responses in rabbits (Alikwe *et al.*, 2012; Ojiezeh, 2013). In view of the potent danger of the result of accumulation of fats (obesity), we therefore, suggest further studies on effects of trace elements on fat accumulation in the body, with a view to determine the dosage level of Mg that will be adequate for normal and healthy living. It might be as result of treatment period, dosage and route of the administration. However, among these three trace elements studied in relation to body - organs weight and pathophysiological challenges, Zn and Cu would be preferred except for the light deposit of fat in Cu supplemented rabbit which may increase with treatment.

## **Conclusion**

Oral supplement of copper sulphate, zinc sulphate and magnesium sulphate for thirty days caused changes in body weight. Increased body-

organ weights ratio of rabbits. The increment in organ weight was obvious in spleen, ovaries, and kidneys. The fat deposit around gastrointestinal tract in female New-Zealand rabbits following Mg and Cu supplementation is worrisome.

### **Conflict of Interests**

The authors do not have a direct financial relationship with the commercial identity mentioned in this paper.

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### **References:**

- Aggett, P. J. (1985) "Physiology and metabolism of essential trace elements: an outline", *Clinical Endocrinology Metabolism*, vol. 14 (3), pp. 513 - 43.
- Alikwe, P.C.N., Ojiezeh, T. I. and Olagboye, S. A. (2012) "Effects of zinc supplement on rabbits performance and growth rate", *Journal of Agriculture and Social Research (JASR)*, vol. 11(2), pp. 46–50.
- Arab, L. (2003)"Biomarkers of fat and fatty acid intake", *Journal of Nutrition*, vol. 133, pp. 925S–932S.
- Brzozowska, M., Kretowski, A., Podkowicz, K., Szmitkowski, M., Borawska, M. and Kinalska, I. (2006) "Evaluation of influence of selenium, copper, zinc and iron concentrations on thyroid gland size in school children with normal ioduria", *Pol. Merkur. Lekarski.*, vol. 20(120), pp. 672 - 677.
- Elliot, J. I. and Bowland, J. P. (1970) "Effects of Dietary Copper Sulfate and Protein on the Fatty Acid Composition of Porcine Fat", *Journal of Animal Science*, vol. 30, pp. 923 - 930.
- Guyton, A. C. and Hall, J. E. (2011) *Textbook of Medical Physiology*, 12<sup>th</sup> ed. Philadelphia, Elsevier inc.
- Huang, Y. L., Wang, Y., Spears, J. W., Lin, X. and Guo, C. H. (2013) "Effect of copper on performance, carcass characteristics, and muscle fatty acid composition of meat goat kids", *Journal of Animal Science*, vol. 91, pp. 5004 - 5010.
- Hurley, L. S. and Swenerton, H. (1966) "Congenital malformations resulting from zinc deficiency in rat", *Pro. Soc. Exp. Bio. Med.*, vol. 123, pp. 692.
- Ibrahim, N. and Mat, I. (1995) "Trace element content in relation to the body weight of the marine bivalve, *Anadara granosa* with special reference to the application of INAA and ICP-AES as analytical techniques", *Journal of Radio analytical and Nuclear Chemistry*, vol.195(1), pp.203- 208.

- National Research Council. (1985) Guides for the Care and Use of Laboratory Animals, National Institutes of Health, Bethesda, Md, USA.
- Nelson, N. (1999) “Metal ion transportation and homeostasis”, *EMBO J.* vol.18 (16), pp. 4361- 4371.
- Ojiezeh, T. I. (2013) “Studies on the effects of cations on antibody stimulation in rabbits”, *Journal of Natural Science Research*, vol. 3(3), pp. 81 - 85.
- Ojiezeh, T. I., Ibeh, N. I. and Okoko, F. J. (2012) “Weight gradient and physiological responses to cation treatment by salmonella enterica infected rabbits”, *African Journal of Clinical and Experimental Microbiology*, vol. 13(3), pp. 98 - 100
- Park, K. (2007) Preventive medicine in obstetrics, Paediatrics and geriatrics. Preventive and Social Medicine. M/s. Banarsidas Bhanot 19th ed. 416 – 532
- Pariza, M. W., Park, Y. and Cook, M. E. (2000) “Mechanisms of action of conjugated linoleic acid: Evidence and speculation”, *Proceed of Society of Experimental Biology and Medicine* vol. 223, pp. 8 - 13.
- Pochon, J. P. (1981) “Zinc and copper replacement therapy a must in burns and scalds in children?” *Progress in Pediatric Surgery*, vol. 14, pp. 151 - 72.
- Solaiman, S.G., Shoemaker, C.E., Jones, W.R. and Kerth, C. R. (2006) “The effects of high levels of supplemental copper on the serum lipid profile, carcass traits, and carcass composition of goat kids”, *Journal of Animal Science*, vol. 84(1) pp. 171 – 177.
- Turin, E.M., Nagle, C.A., Lahoz, M., Torres, M., Turin, M., Mendizabal, A. F. and Escofet, M. B. (1997) “Effects of a copper-bearing intrauterine device on the ovarian function, body weight gain and pregnancy rate of nulliparous heifers” *Theriogenology*, vol. 47 (7), pp. 1327 - 1336.
- Zeme, B. S., Kawchak, D. A., Fung, E. B., Ohene – Frempong, K. and Stallings, V. A. (2002) “Effect of zinc supplementation on growth and body composition in children with sickle cell disease”, *America Journal of Clinical Nutrition*, Feb., vol.75 (2), pp.300 - 307.
- Zlotkin, S. H., Atkinson, S. and Lockitch, G. (1995) “Trace elements in nutrition for premature infants”, *March*. Vol. 22 (1), pp. 223 - 40.