PHYSIOPATHOLOGY DUE TO COPPER INCRESE IN RABBIT DIETS (*Oryctolagus cuniculus*).

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Summary

Copper is an enzymatic cofactor, essential for Fe usage, connective tissue formation, pigmentation and energy production. The objective of this study was to analyse the physiopathology of renal and hepatic damage in rabbits fed alfalfa with high copper content from a rabbit farm in the State of Mexico, from which twelve rabbits were sampled, taking from the auricular vein 4 mL of blood with and without anticoagulant in different periods to evaluate physiological parameters: hematocrit, total proteins, ALT (alanine aminotransferase), AST (aspartate transferase), GGT (gamma glutamyl transferase), urea and creatinine. The first samples were taken when the rabbits aged 8 weeks, and then 15, 30, 45 and 60 days afterwards. Each sample was centrifuged and divided for analysis in the laboratory, comparing the results with reference values. Initial hematocrit found was 43 ± 2.50% and 37 ± 14.57 % at 60 days; total protein concentration started in 7.2 ± 0.49 and decreased to 5.83 ± 0.15g/dL 45 days after; initial ALT levels were 48 ± 13.89 UL increasing to a final level of 64 ± 4.16 UL; average GGT levels were 10 ± 2 UL; regarding AST levels on day 45 were 33 ± 4.73 increasing to 51 ± 23.12 UL on day 60; urea increased from 0.3 ± 0.10 mmol/L up to 27 ± 1.65 mmol/L on day 60; initial creatinine levels were 90.18 ± 6.47 µmol/L increasing up to 121 ± 6.53 µmol/L in the last period. The parameters obtained show that rabbit exposed to copper suffer physiological changes which alter erythrocyte, renal and hepatic physiology.

Key words: Copper, rabbit, hematocrit, total proteins, clinical enzymology, liver, kidney.

Introduction

Copper is an essential body mineral, which is part of the enzymes ferro-oxidase, cytochrome oxidase, superoxide dismutase, amine oxidase, uricase and dopamine-β-hydroxylase and some others. Therefore, it should be present in low quantities as micronutrient, needing it for growth, energy production, pigmentation, connective tissue formation, blood cell production and for functional and structural maintainance of nervous cells. Copper from feed reaches the intestine where it is absorbed. Only a small quantity is absorbed, and the rest (more than 90-95%), is eliminated through feces. The absorbed copper passes to blood and is transported to the liver, where it accumulates. Copper as the rest of the essential minerals may be toxic when concentrations exceed the ones on the diet. Copper intoxication phases include: copper accumulation first, lasting days to months depending on the quantitiy in the diet, the absorption percentage and factors which accelerate its deposit and mobilization with no effect on the animal's behavior. During the second phase, known as hemolytic crisis, the apoptosis of hepatic cells and copper liberation to circulation occurs. This circulating copper destroys red blood cells (up to 60%) liberating hemoglobin. The toxic effect of this element provokes hemolysis, hepatic necrosis and gastrointestinal hemorrages. When chronic intoxications occur, respiratory irritation, gastrointestinal alterations and contact dermititis may be present. Systemically, hemolytic anemia, hepatic degeneration, renal, cerebral and visual alterations are originated. The objective of this research was to evaluate the hepatic and renal function in rabbits with exposure to copper in the diet and to document the effect of high levels of this micronutrient.

Material and Methods

A New Zealand rabbit farm with 100 fattening animals was chosen, due to spontaneous diarrheic cases, weakness and mortality of 2-3%. Feeding was *ad libitum* with commercial Purina® (16 % protein, 3% fat, 17 % fiber, 10 % ashes, 12% de humidity, 42.5 % nitrogen free extract, 1 % calcium and 0.55 % phosphorus) and drinking water. Green alfalfa was added, which was

cultivated and fertilized with poultry manure and well water, in which an atomic absorption spectrophotometric analysis for copper was 19,2 mg/kg. Twelve rabbits for human consumption were sampled in different periods, starting at age 8 weeks, and then 15, 30, 45 and 60 days afterwards, collecting 4 mL of blood in Vacutainer tubes with and without anticoagulant (Vacutainer, SST: Becton-Dickinson, U.S.A.), taken from the auricular vein, to evaluate the following physiologic parameters: hematocrit, total proteins, ALT, AST, GGT, urea and creatinine. The samples were centrifuged and divided to analyse them in the laboratory, and the results were compared with reference values for rabbits. Hematocrit was determined using heparinized capilar tubes by centrifugation at 5000 rpm, for 5 minutes and total protein through refractometry (Veterinary Refractometer 10436, Reichert® g/%). Analite determinations were quantitatively obtained through spectrophotometry, by colorimetric method for ALT and AST through the modified method without pyridoxal phosphate, measuring absorbance at 340 nm; for GGT the Szazs/Persijn method was used; measuring absorbance at 405 nm; urea was measured through urease/GLDH enzymatic method at 340 nm; and creatinine through colorimetric method based on the creatinine reaction with picric acid in alcaline medium at 510 nm (Clinical chemistry: Instrumentation laboratory®).

Results and Discussion

Hematocrit significantly decreased from 43 ± 2.50% to 37 ± 14.57 % in the fourth period, and total proteins just showed a slight decrease from 7.2 ± 0.49 to 5.83 ± 0.15g/dL in the third one. Due to the high affinity of copper to sulphur or nitrogen, it forms very stable coordination complexes. It joins to thyolic groups in hemoglobin and in the cellular membrane, provoking cellular lysis. By glutathione reductase inhibition and hyperstimulation of the hexose monophosphate via, it depletes the glutathione content, and also inhibits cellular respiration. The reported signology in rabbits is probably associated to the alteration of the parameters which were evaluated. The alteration of blood cell production and viability, with the cell pack observed can be associated with anemia, and an adequate protein utilization in the metabolic processes of

the rabbits (Thrall *et al.*, 2006). ALT initial value was 48 ± 13.89 U/L, in period 1, 49 ± 15.25 U/L, in period 2, 60 ± 22.50 U/L, stabilizing in period 3 to 46 ± 19.08 U/L, with 64 ± 4.16 U/L in the last period; GGT values were reported as normal; AST values decreased in period 3 to 33± 4.73 U/L, maintaining normal values in the rest; urea progressively increased up to 27 ±1.65 mmol/L, with an initial value of 0.3 ± 0.10 mmol/L; creatinine initial values were 90.18 ± 6.47 µmol/L and increased up to 121 ± 6.53 µmol/L in the last period (Table 1).

Table 2. Hepatic and renal functionality parameters in rabbits (*Oryctolagus cuniculus*) from a farm in the State of Mexico

PARAMETER	Day 0	Day 15	Day 30	Day 45	Day 60	Reference values
HEMATOCRIT (%)	43±2.50	36±5.47	46±1.00	38±2.08	37±14.57	40.5 ± 2.11
TOTAL PLASMA PROTEINS (g/dL)	7±0.49	6±0.26	6±0.33	5±0.15	6±0.46	5.7 ± 0.6
ALT (U/L)	48±13.89	49±15.25	60±22.50	46±19.08	64±4.16	79 U/L
AST (U/L)	45±15.03	41±4.51	40±7.29	33±4.73	51±23.12	47 U/L
GGT (U/L)	10±2.56	10±2.23	13±2.07	8±3.21	13±3.21	9 U/L
UREA (mmol/L)	0.3±0.10	8±1.40	8±1.60	19±1.82	27±1.65	3.45 ± 0.85
CREATININE (µmol /L)	90±6.47	98±14.34	118±28.50	94±7.70	121±6.53	70.7-227.2

The report on the copper content in the alfalfa may be considered high, therefore assuming that the absorption for this species is also high. Moreover, the parameter evaluated in this study corroborate that some of them fluctuate and tend to progressively increase, and that they may be related with structural and functional damage of liver and kidney (Thrall *et al.*, 2006). Copper has been referred as highly toxic to inferior organisms and low toxic for superior ones. Two genetic alterations have been documented for copper metabolism: Wilson disease (caused by ceruloplasmin deficit) in which hepatic and brain degeneration are produced and Menke's syndrome, where brain degeneration caused by copper deficiency occurs. Copper accumulates in the liver with no apparent intoxication signs, up to a point where the accumulation capacity of the organ is overflowed. When this happens, necrosis of the hepatocytes occur, with sudden high

copper concentration in blood, unchaining clinical intoxication, followed by an hemolytic crisis in ruminants (destruction of red blood cells).

Conclusions

The parameters found show that the organisms with copper exposure suffer physiologic changes which may permanently alter renal and hepatic functionality, interfering with the correct animal development, limiting nutrient absorption and growth.

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