WORLD RABBIT SCIENCE, VOL 10 (4), 167-170

TECHNICAL NOTE

CONCENTRATION OF COPPER IN MUSCLES, LIVER, HAIR AND FAECES OF GROWING RABBITS FED DIET SUPPLEMENTED WITH COPPER SULPHATE

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ABSTRACT: The supplementation of diets for rabbits with copper has been restricted in the EU to 35 mg/kg. In our experiment, quadruple concentration of Cu added to a pelleted diet as $CuSO_4.5H_2O$ was used to investigate effect of Cu feeding on Cu concentration in muscles, liver, hair and faeces of Hyplus® rabbits. The main ingredients of the basal diet were alfalfa meal, wheat bran, sunflower meal, oat, barley, sugarbeet pulp and soyabean meal. Six rabbits, 35 days of age at the beginning of the experiment, were fed basal diet (containing 10 mg Cu/kg), and six rabbits were fed Cu–supplemented diet. Cu concentration in faeces was measured weekly. Rabbits were slaughtered at the age of 87 days. Cu concentrations in muscles, liver, hair and faeces were assayed by the atomic absorption spectrometry. The highest concentration of Cu was found in liver of Cu–fed rabbits: 118.5 ± 31.8 mg/kg (4.6 ± 0.5 mg/kg in controls). The Cu concentration in meat of Cu–fed rabbits was very low: 0.48 ± 0.05 and 0.37 ± 0.04 in hindleg and loin, respectively. Corresponding Cu concentrations in control rabbits were 0.48 ± 0.05 and 0.35 ± 0.04 mg/kg. Hair of Cu–fed and control rabbits contained 9.55 ± 0.46 and 9.14 ± 1.05 mg Cu/kg, respectively. On average, faeces of Cu–fed and control rabbits contained 273 and 15.3 mg Cu/kg, respectively. Faecal excretion is thus probably the main route of Cu output in Cu–fed rabbits. On the basis of our results, we conclude that Cu-fed rabbits accumulated Cu in the liver. No substantial Cu retention in the meat of Cu-fed supplemented rabbits was observed.

Key words: Copper retention, Mineral metabolism, Copper supplementation, Growing rabbits

Résumé : La supplémentation en cuivre pour l'alimentation des lapins a été limitée à 35 mg/kg dans l'union européenne. Dans cette étude, la supplémentation en cuivre (à partir de $CuSO_4.5H_2O$) dans l'alimentation des lapins (Hyplus®) a été quadruplée, afin d'étudier l'effet du cuivre ingéré sur sa concentration dans la viande, le foie, les poils et les faeces. Les ingrédients principaux de l'aliment de base sont: la luzerne déshydratée, le son de blé, le tourteau de tournesol, l'avoine, l'orge, la pulpe de betterave et le tourteau de soja. Six lapins, âgé de 35 jours en début d'essai, ont été nourris avec l'aliment de base (10 mg Cu/kg), et 6 autres ont été nourris avec l'aliment supplémenté en cuivre. La concentration en cuivre dans les faeces a été mesurée chaque semaine. Les lapins ont été sacrifiés à 87 jours d'âge. La concentration en cuivre (Cu) dans la viande, les poils, les faeces a été analysée par spectrométrie d'absorption atomique. La concentration en Cu la plus forte est dans le foie des lapins nourris avec l'aliment supplémenté en Cu: 118,5 ± 31,8 mg/kg (4.6 ± 0.5 mg/kg pour les témoins). La concentration en Cu dans la viande des lapins supplémentés est très faible : 0,48 ± 0,05 et 0,37 ± 0.04 resp. dans la cuisse et le rable. Les valeurs correspondantes chez les lapins témoins sont: 0,48 ± 0,05 et 0,35 ± 0,04 mg/kg. Les poils des lapins supplémentés et témoins contiennent respectivement 9,55 ± 0,46 et 9,14 ± 1,05 mg Cu/kg. En moyenne, la concentration en cuivre des faeces est 273 et 15,3 mg Cu/kg, respectivement chez les lapins supplémentés et témoins. L'excrétion faecale est probablement la voie principale d'élimination du cuivre excédentaire chez les lapins supplémentés. En conclusion, les lapins supplémentés en cuivre accumulent du cuivre dans le foie. Il n'y a pas de rétention significative du cuivre dans la viande des lapins supplémentés.

INTRODUCTION

Copper is an essential component of many enzymes, on the other hand it is a heavy metal dangerous at high intake for human health. The nutritional requirement of rabbits for copper is about 10 mg/kg (MATEOS and DE BLAS, 1998). Copper added at much higher dose, usually at 100 – 300 mg/kg, has been recognized by several authors as a growth promoting substance (FEKETE *et al.*, 1988; ONIFADE and ABU, 1998; ABOUL-ELA *et al.*, 2000). Beneficial effects of copper added to a diet at this concentration exist also in poultry (e.g. PESTI and BAKALLI, 1996; SKŘIVAN *et al.*, 2000), and in pigs (e.g. EDMONDS *et al.*, 1985; ZHOU *et al.*, 1994). In our previous experiment, copper added as copper sulphate pentahydrate at 150 mg Cu/kg to a diet of growing rabbits non-significantly increased weight gain by 9.1%, and (in combination with vitamin E addition) significantly decreased the cholesterol concentration in the loin and hindleg meat by 13.6 and 17.9%,

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respectively (SKŘIVANOVÁ *et al.*, 2001). In the EU the maximum concentration of copper allowed in rabbit diets has been restricted to 35 mg/kg. In other parts of the world copper remains an alternative to in-feed antibiotics, which does not contribute to antibiotic resistance transfer to pathogenic bacteria.

The level of Cu intake that has been recommended as safe and adequate is 1.5 – 3.0 mg per day for adult humans (National Research Council, 1989). The aim of our study was to investigate the deposition of copper in the meat and liver of rabbits fed a diet supplemented with copper at 140 mg Cu/kg. Our hypothesis was that the acceptable daily Cu intake would not be exceeded in humans consuming these rabbits. In addition, the deposition of copper in hair and its excretion through faeces in rabbits fed supplemental copper were investigated.

MATERIALS AND METHODS

Twelve Hyplus® rabbits, 35 days of age at the beginning of the experiment, were used. Rabbits, purchased in a commercial rabbitry, were housed individually in stainless mesh cages (0.22 m^2), equipped with a nipple drinker and an outside placed feeder. The rabbits had *ad libitum* access to feed and water. Six rabbits were fed a control pelleted diet (Table 1), the remaining six rabbits were fed the same diet supplemented with 0.55 g of $CuSO_4.5\ H_2O$ per kg (140 mg Cu/kg). Rabbits were housed in environmentally-controlled experimental facility. Environmental conditions were: temperature 16 ± 1°C, relative humidity 65%, 12 h light: 12 h dark daily photoperiod cycle. Faeces of all rabbits were sampled five times, in weekly intervals.

Rabbits were slaughtered at the age of 87 days. Samples of tissues (loin and hindleg meat, liver) were homogenized and dried at 105°C. Also faeces and hair were dried at 105°C. All samples were ashed at 450°C, extracted by diluted HNO_3 (1 : 3), and Cu in extracts was determined by the atomic spectrometry, using a Solaar M6 instrument (TJA Solutions, Cambridge, UK). Copper was assayed also in the control diet. Dry matter, crude protein, crude fibre, fat and ash concentrations in the diets were determined by

Table 1: Ingredients and determined chemical composition of control rabbit diet.

Ingredients (%)		**Nutrients (g/kg as fed)**	
Alfalfa meal	30	Dry matter	914
Wheat bran	22.5	Crude protein	175
Sunflower meal	17	Crude fibre	194
Oat	9	Crude fat	26
Barley	8	Ash	80
Sugarbeet pulp	6	Cu	0.01
Soyabean meal	3		
Rapeseed oil	1.5		
Vitamin supplement	1		
Limestone	1		
Dicalcium phosphate	0.5		
Salt	0.5		

Table 2: Concentration of copper (mean±S.D.) (mg/kg wet weight) in hindleg and loin meat, liver, and hair of rabbits receiving control diet and diet supplemented with copper sulphate.

Sample	Diet		*P*-value
	Control	Control +140 mg Cu/kg	
Hindleg meat	0.48 ± 0.05	0.48 ± 0.05	NS
Loin meat	0.35 ± 0.04	0.37 ± 0.04	NS
Liver	4.62 ± 0.49	118.5 ± 31.8	< 0.001
Hair	9.14 ± 1.05	9.55 ± 0.46	NS

NS: not significant

standard AOAC (1980) procedures. The *t*-test was used to evaluate the effect of copper supplementation.

RESULTS AND DISCUSSION

Table 2 presents data on concentration of copper in the muscles of loin and hindleg, in liver, and hair of control and treated rabbits. Both groups did not differ in copper concentrations in meat of hair. The copper concentration in the liver of Cu-fed rabbits, however, was twenty-five times higher than that in the liver of control rabbits (118.5 *vs* 4.6 mg Cu/kg; $P< 0.001$). Very high concentration of copper was also present in faeces of treated rabbits (on average 273 mg Cu/kg wet weight). Copper concentration in faeces of control rabbits was only 15.3 ± 1.3 mg/kg (Table 3).

ABO-EL-EZZ *et al.* (1996) found 19.5 ± 4.8 mg Cu/kg in fore leg muscles of rabbits fed a diet supplemented with 180 mg Cu/kg, and 7.9 ± 0.9 mg Cu/kg in the muscles of rabbits fed a control diet. On the contrary, SKŘIVANOVÁ *et al.* (2001) found 0.94 and 0.80 mg Cu/kg in the loin and hindleg muscles of rabbits fed a diet supplemented with 150 mg Cu/kg, but 1.06 and 0.88 mg Cu/kg, respectively, in the muscles of control rabbits fed the same diet without supplemental copper. FALANDYSZ (1991) found variable concentrations of copper (0.52 to 7.3 mg/kg) in meat of poultry, rabbit and sheep. The results presented here are in agreement with our previous findings (SKŘIVANOVÁ *et al.*, 2001), and support hypothesis that excessive dietary copper accumulates in the liver (probably also in the kidney), but not in muscles. HULLÁR *et al.* (1996) observed a minimal contamination of rabbit meat by Cd, Pb and Hg in rabbits fed carrot diets with high concentrations of

Table 3: Concentration of copper copper (means±S.D.) (mg/kg wet weight) in faeces of rabbits receiving control diet and diet supplemented with copper sulphate.

Age (week)	Diet		*P*-value
	Control	Control + 140 mg Cu/kg	
8	14.2 ± 1.3	265 ± 28	< 0.001
9	16.5 ± 2.0	254 ± 42	< 0.001
10	17.1 ± 1.5	265 ± 40	< 0.001
11	15.2 ± 1.0	286 ± 40	< 0.001
12	13.7 ± 0.9	295 ± 23	< 0.001

heavy metals. These authors reported high concentrations of heavy metals in the liver and kidney tissue, and identified feacal excretion as the main route of Cd, Pb and Hg elimination from the body. In humans, urinary losses of copper are low. Excess hepatic Cu is exported into bile and eliminated from the body via faeces (FAILLA, 1999). Thus, high hepatic and faecal concentrations of copper found in this study are not surprising. Contrary to our expectation, the copper concentration in hair of rabbits was not significantly influenced by dietary copper concentration.

The Czech standard allows 5 mg of Cu per kg of meat. In spite of pharmacological concentrations of Cu in the diet of treated rabbits, concentrations of Cu in muscles were low, not increased above the acceptable level. On the other hand, liver should not be recommended for human consumption if rabbits are fed high doses of Cu, or raised in highly polluted regions.

Acknowledgements: This study was financed by the Ministry of Agriculture of the Czech Republic (project No. MO2 – 99 – 04).

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