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HIGH DIETARY LEVELS OF ZINC FOR YOUNG RABBITS

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ABSTRACT

The effects of orally administered zinc from inorganic or organic sources on selected parameters of meat quality were the priority of this study. A total of 96 weaned rabbits (35th day of age, both male and female) were divided into 4 groups (control C and 3 experimental groups – 1EG, 2EG and 3EG) with 24 animals in each group. Maternal albinotic line (crossbreed New Zealand White, Buskat Rabbit, French Silver) and paternal acromalictic line (crossbreed Nitra‘ Rabbit, Californian Rabbit, Big Light Silver) were used. The feed mixture was additionally administered as follows: in 1st experimental group 1EG by a dose of 27.47 g $ZnSO_4.H_2O$ (zinc sulphate monohydrate), in 2nd group (2EG) by a dose of 38.46 g Glycinoplex-Zn and in 3rd group (3EG) dose of 66.67 g Bioplex-Zn, per 100 kg each. They were fed with complete granulated mixtures *ad libitum* and had free access to water via a nipple drinker. Dietary supplementation of rabbit with zinc was carried out to determine its effects on growth of live weight and consumption of feed per unit of live weight growth. On 91st day of age (6 weeks after all experimental procedures), 6 animals from each group were slaughtered and sampled for testing in the morning hours. Meat quality was analyzed from each sample of *Musculus longissimus dorsi* (MLD) (50 g) for parameters characterizing the content of nutrients (content of water, proteins, fat, amino acids and fatty acids composition) and processing technology parameters (electric conductivity, pH, colour). The amino acids and fatty acids contents noted in this study indicate statistically insignificant changes ($p \leq 0.05$). This study suggests that lean rabbit meat could be a high quality protein source due to its well-balanced essential amino acid composition. The growth rate in all groups was independent on zinc treatment. A weak influence of Glycinoplex-Zn on animal health was also noted. Zinc supplementation raises levels of cholesterol, water holding capacity and energy value, and lowers the value of pH in *longissimus dorsi* muscle compared to rabbits fed the control diet. Supplementation with Glycinoplex-Zn (100 mg of zinc) evoked a 25 % mortality rate respectively in comparison to 8.3 % of the Bioplex-Zn supplemented with frequent *Pasteurella* infections. These effects were not observed in rabbits fed with other diets.

Key words: rabbits; zinc; meat quality

INTRODUCTION

Zinc (Zn) is both an essential nutrient and a possible pollutant in animal production system. While it is generally supplemented at low levels in animal diets (less than 200 $mg.kg^{-1}$ in complete feeds), it is under scrutiny due to potential accumulation in the environment. This explains why international regulations strictly limit maximum supplementation levels in animal feeds. The role of micro-minerals in health cannot be over emphasized; zinc has been a modifier of wide spectrum of biological activities. Its deficiency has been related to various dysfunctions and alterations of normal cell metabolism. In this study, supplementation of rabbits with zinc salt was conducted to determine its effects on reproductive performance and growth rate following improvement in the quality and quantity of non-traditional meat as a source of protein for the

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consumers (Underwood, 1977; Alikwe *et al.* 2011). The role of zinc in the animal organism has begun to gain special attention. Zinc participates actively in protein synthesis and carbohydrate metabolism. The discovery that the enzyme carbonic anhydrase contains 0.33 % of zinc in its molecule is considered the first acceptable explanation of Zn mechanism of action. After that, many other enzymes have been identified to contain zinc: alcohol dehydrogenase, carboxipeptidase and DNA-polymerase, the latest being fundamental in cell division process. This mineral stabilizes the quaternary structure of enzymes; large quantities of zinc were found to provide stability to the structures of RNA, DNA and ribosomes (Prask and Plocke, 1971, quoted by Mc Dowel, 1992). Zinc requirement for rabbits is 30 – 60 mg.kg^{-1} dry matter, with suggestion of higher levels for breeders (Mateos and Blas, 1998). It is also essential in cell division, synthesis and stability of DNA (Evenson *et al*., 1993), as well as in cellular differentiation. Animals need microelements in small quantities, and these microelements play an important role in virtually all physiological and biochemical processes, from bone structure to maintaining the structure of proteins and lipids. Microelements are provided to animals in food, by special supplementation (premixes) or in water. In the intensive production, their addition is obligatory, since it has been the only way to provide them in sufficient quantities required for optimum health and production results (Pajtáš *et al*., 2009; Chrastinová *et al*., 2014; Gralak and Chrenková, 2014).

Minerals activate enzymes and they are essential cofactors of metabolic reactions, and function as carriers of proteins, regulate digestion, respiration, water balance, muscle response, the neural transmissions, influence and maintain skeletal strength, balance pH, and even mental balance, protect against disease, act as antagonists or synergists of other elements and play a vital role in the resistance, adaptation and evolution of new races and lines (Anke and Szentmihalyi, 1985; Haenlein, 1987).

Regardless of the fact that certain microelements are present in sufficient quantities in food, subclinical or clinical symptoms of their deficit appear, because their availability varies, or the microelement is present in a form that cannot be used. It was established that the presence of certain substances in food (phytic acid and oxalic acid), as well as interaction with other nutrients in the digestive tract influences resorption mechanisms. Resorption of microelements is not dependent only on their content in food, but also on the animal‘s age, on electrochemical reactions in the intestine, and on the form of the microelement. Salts of minerals are most frequently used, namely oxides, carbonates, chlorides, and sulphates. Today, in addition to inorganic forms of minerals, the use of so-called „chelate“ forms, i.e. organically bonded microelements is becoming more frequent. The aim of this study was to reveal the effects of orally administered zinc from inorganic or organic sources on selected parameters of meat quality.

Table 1: Composition and nutrient content of granulated diet for growing rabbits

Ingredients	%	Chemical analysis	Original matter (g.kg^{-1})
Lucerne meal	36	Crude protein (N*6,25)	177.99
Extracted sunflower meal	5.5	Crude fibre	146.97
Extracted rapeseed meal	5.5	Fat	36.08
Wheat bran	9	Ash	97.32
Oats	13	Starch	129.05
Malt sprouts	15	Organic matter	847.49
DDGS	5	Acid detergent fibre (ADF)	185.13
Sodium chloride	0.3	Neutral detergent fibre (NDF)	315.49
Mineral and vitamin mixture*	1.7	Calcium	9.73
Barley grains	8	Phosphorus	6.94
Limestone	1	ME (MJ.kg^{-1})	11.35

*Premix contains per kg: calcium, 6.73 g; phosphorous, 4.13 g; magnesium, 1.90 g; sodium, 1.36 g; potassium, 11.21 g; iron, 0.36 g; copper, 0.03 g; selenium, 0.2 mg. Vitamin mixture provided per kg of diet: Vitamin A 1500000 IU; Vitamin D3 125000 IU; Vitamin E, 5000 mg; Vitamin B1, 100 mg; Vitamin B2, 500 mg; Vitamin B6, 200 mg; Vitamin B12, 0.01 mg; Vitamin K3, 0.5 mg; biotin,10 mg; folic acid, 25 mg; nicotinic acid, 4000 mg, choline chloride, 100000 mg; DDGS: dried distillers grains with solubles

MATERIAL AND METHODS

A total of 96 weaned rabbits (35th day of age, both male and female) were divided into 4 groups (control C and 3 experimental groups – 1EG, 2EG and 3EG) with 24 animals (in a replicated 6 x 4) in each group. The rabbits of meat line M91, maternal albinotic line (crossbreed New Zealand white, Buskat rabbit, French silver) and paternal acromalictic line (crossbreed Nitra's rabbit, Californian rabbit, Big light silver) were used in this experiment. The experiment lasted 48 days. Rabbits were kept in the standard cages, 2 animals per cage. A cycle of 16 h of light and 8 h of dark was used throughout the experiment. Temperature and humidity in the building were recorded continuously by a digital thermograph positioned at the same level as the cages. Heating and forced ventilation systems allowed the building air temperature to be maintained within 22 ± 4 ˚C throughout the experiment. Relative humidity was about 70 ± 5 %. The rabbits were fed with a commercial diet (pellets of 3.5 mm in diameter). The ingredients and chemical composition of this diet is presented in Table 1.

The feed mixtures for other testing groups (1EG, 2EG and 3EG) were additionally administered before homogeneity of feed mixture: in 1st experimental group by a dose of 27.47 g $ZnSO_4.H_2O$, in 2nd group (2EG) by a dose of 38.46 g Glycinoplex-Zn and in 3rd group (3EG) by a dose of 66.67 g Bioplex-Zn per 100 kg. They were fed with complete pelleted diets *ad libitum* (Table 2). Animals had free access to water via a nipple drinker. In this study, institutional and national guidelines for the care and use of animals were followed, and all experimental procedures involving animals were approved by the ethical committee. The ME content was calculated by the equation of Wiseman *et al*. (1992). Chemical analyses were conducted according to AOAC (1995) with the considerations given by Gidenne *et al*. (2001) for dry matter (DM), crude protein (CP), crude fibre (CF), crude fat, nitrogen free extract, ash and organic matter. Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were analysed sequentially (Van Soest *et al*., 1991) with a thermo stable amylase pre-treatment and starch according to the alpha-amyloglucosidase method.

Body weight and feed consumption were registered weekly. On 91st day of age (6 weeks after all experimental procedures), 6 animals from each group were slaughtered and sampled for testing in the morning hours. After electro-stunning (90 V for 5 s), rabbits were slaughtered in an experimental slaughterhouse by cutting the carotid and jugular veins, bleeding out; the MLD samples were separated by removing the skin and connective tissue chilled and stored for 24 h at 4 °C until physico-chemical analysis. The ultimate pH was determined after 24 h (*post mortem*) using a Radelkis OP-109 (Jenway, England) with a combined electrode penetrating 3 mm into samples. The electrical conductivity ($\mu S.cm^{-1}$), defined as locations of muscles, was evaluated using PMV 51 (Tecpro GmbH, Germany), colour characteristics were expressed by CIE L*a*b system

Table 2: Performance of rabbits in response to dietary supplementation with zinc from inorganic or organic sources ($\bar{x}$ ± SD)

Parameters (n = 24)	C	1EG	2EG	3EG	C	1EG	2EG	3EG
	Adaptation period				Experimental period			
Feed intake g	91.73	89.35	89.74	92.74	133.93	132.66	130	132.83
Initial weight g	1316 ±103	1362 ±59	1374 ±156	1335 ±14	1637 ±119	1633 ±33	1663 ±183	1638 ±93
Final weight g	1637 ±119	1633 ±33	1663 ±183	1638 ±93	2971 ±160	3004 ±229	3049 ±207	2954 ±189
Daily weight gain $g.day^{-1}$	41.61	38.37	41.37	43.36	31.76	32.63	33.00	31.34
Feed conversion ratio $g.g^{-1}$	2.20	2.31	2.16	2.14	4.23	4.08	4.20	4.26
Carcass yield %	-	-	-	-	59.24 ±0.78	59.41 ±1.59	60.12 ±0.45	58.37 ±3.38
Mortality (n)	0	0	1	2	0	0	5	0

Control-C; 1EG – with $ZnSO_4.H_2O$; 2EG – with Glycinoplex-Zn; 3EG – with Bioplex-Zn

(lightness-L*, 0: black and 100: white), (redness and greenness-a*; yellowness and blueness-b*) using a Lab. Miniscan. Lightness measurements at room temperature were also done. The content of water, protein and fat were estimated using an INFRATEC 1265 (Germany) spectroscope and expressed in $g.100.g^{-1}$; and from these values, the energy value was calculated:

EV ($kJ.100.g^{-1}$) = 16.75 x protein content + 37.65 x fat content.

The water holding capacity was determined by the compress method at constant pressure (Hašek and Palanská, 1976). The fatty acid (FA) composition of MLD samples were determined (Ouhayoun, 1992) by gas chromatography of fatty acid methyl ester (FAME) on GC 6890 N (Agilent Technologies, Switzerland). Results were expressed as percentages of total fatty acids. Fatty acid composition varies a lot and is expressed as share of SFA (saturated fatty acid), MUFA (monounsaturated fatty acid), PUFA (polyunsaturated fatty acids), P/S and n6/n3 index. The amino acids composition of diet was analyzed by ion-exchange chromatography on AAA (Ingos Prague, Czech Republic) after acid hydrolysis with 6 M HCl, methionine and cystine after oxidation hydrolysis. Weight of feed mixture was checked daily and average daily weight gain and feed conversion were calculated mathematically as well as mortality at the end of the experiment.

The results were expressed as mean ± standard deviation (SD); statistical evaluation of the results was performed by the one-way ANOVA and Tukey test for multiple comparisons at the level of significance $p \leq 0.05$.

RESULTS AND DISCUSSION

The study was performed in the National Agricultural and Food Centre, Research Institute for Animal Production Nitra. Among the experimental groups no significant difference was noted in feed intake, feed conversion ratio and carcass value in the fattening experiment. Results regarding the zootechnical parameters are shown in Table 2. The study was divided into two phases: adaptation period (35^{th} day to 49^{th} day of age) and experimental period (49^{th} day to 91^{st} day of age).

Daily weight gain from weaning (on 35^{th} day) reached 38.75 vs. 43.33 g daily. The second phase over the study period at slaughtering age 91^{st} day reached 31.34 vs. 33.0 g daily weight gain; it was influenced by inorganic or organic sources of zinc supplementation in the rabbits' diet in groups 1EG and 2EG was compared with CG and 3EG. Increase in average body weight gain (from 0.89 to 1.24 g) was noted in the rabbits of group 1EG and 2EG compared to CG.

The average carcass dressing out percentage (58.37 vs. 60.12 %) was calculated. Results of selected meat quality parameters (content of water, content of proteins, fat and amino acids, fatty acids, electric conductivity, pH and colour) are presented in Table 3. Zinc supplementation raises levels of cholesterol, water holding capacity and energy value, and lowers the value of pH in *longissimus dorsi* muscle compared to rabbits fed the control diet. The fatty acids composition in MLD muscles is shown in Table 3. The rabbit muscles are a low-fat meat. The intramuscular lipid was characterized by the highest percentage of monounsaturated fatty acids (MUFA) (50.17 vs. 52.56 %). In this study the intramuscular lipids in the MLD muscles were also characterized by a higher percentage of saturated (SFA) (39.36 vs. 39.93 %) and lower percentage of polyunsaturated fatty acids (PUFA) (10.94 vs. 11.37 %). The higher percentage of MUFA in the intramuscular fats was determined in fine trial. The amino acid composition of MLD muscles is shown in Table 4. Rabbit protein contained a high amount of lysine, leucine, arginine, isoleucine, histidine, valine, threonine, phenylalanine, methionine and cystine in decreasing amounts. Moreover, the sequence of other amino acids is similar to the sequence of amino acids in other meats. The essential amino acid composition is one of the most important nutritional qualities of protein. The highest content of lysine, leucine, valine, threonine and sum of essential amino acids was specified in group 2EG with supplementation of Glycinoplex-Zn ($p \leq 0.05$). Nowadays, histidine is considered to be an essential amino acid because of the detrimental effects on haemoglobin concentrations (Report of a Joint WHO/ FAO/ UNU Expert Consultation, 2007). According to all of the detected amino acid scores, the protein in MLD muscle was well-balanced in essential amino acid composition and is of high quality. According to this study, lean rabbit meat could be considered a high quality protein source due to its well-balanced essential amino acid composition. No recent trials on the zinc requirements of rabbits could be traced in the literature, but levels of use vary between 25 and 60 $mg.kg^{-1}$, with the higher values proposed for does and bucks. Practical commercial diets contain a wider range of zinc (40–140 $mg.kg^{-1}$). Zinc oxide is the most commonly used source because it is less reactive and has a higher zinc concentration than sulphate and carbonate salts. No differences in zinc bioavailability between inorganic and organic sources have been reported in rabbits (Guimaraes and Motta, 2000; De Blas and Wiseman, 2010).

Because of zinc's environmental impact, the maximum level allowed in the EU for rabbit feeds is 150 mg.kg^{-1}. In addition, the adverse effect of high zinc intake on copper availability has to be considered (Maret and Sandstead, 2006). We agree with the proposal of EU for the Zn level in rabbits' diet, although in organic form of Zn we suggest to decrease the level in diet by about 20 %.

Table 3: The effect of dietary zinc supplementation on selected physico-chemical characteristics of MLD muscles 24 h *post mortem* ($\bar{x} \pm$ SD)

Characteristics (n = 6)	Control-C	1EG - with $ZnSO_4.H_2O$	2EG - with Glycinoplex- Zn	3EG - with Bioplex- Zn
Water g.100.g^{-1}	74.61 ± 0.44	74.89 ± 0.19	74.74 ± 0.55	74.64 ± 0.44
Protein g.100.g^{-1}	23.49 ± 0.43	23.31 ± 0.19	23.51 ± 0.27	23.67±0.57
Fat g.100.g^{-1}	0.92 ± 0.23	1.04 ± 0.13	0.89 ± 0.12	0.91 ± 0.21
Colour L	53.54 ± 1.94	52.21 ± 3.09	50.25 ± 2.98	48.88 ± 2.54
Electrical conductivity	1.41 ± 0.75	1.08 ± 0.40	2.50 ± 1.33	1.73 ± 0.85
Cholesterol g.100.g^{-1}	0.25 ± 0.06	0.30 ± 0.05^{a}	0.26 ± 0.05	0.27 ± 0.08
pH_{24}	6.16 ± 0.06	6.09 ± 0.07	6.07 ± 0.08	6.09 ± 0.06
Water holding capacity	26.33 ± 3.84	29.60 ± 3.08	31.03 ± 4.06	30.77 ± 3.52
EV(kJ.100 g^{-1})	427.15 ± 5.68	429.65 ± 4.55	427.30 ± 8.82	430.79 ± 8.69
		Fatty acids (% of total FA)		
Lauric (C12:0)	0.05 ± 0.007	0.05 ± 0.01	0.05 ± 0.01	0.05 ± 0.01
Myristic (C14:0)	1.24 ± 0.04	1.26 ± 0.04	1.24 ± 0.03	1.24 ± 0.03
Palmitic (C16:0)	24.33 ± 0.24	24.60 ± 0.09^{ad}	24.44 ± 0.16	24.33 ± 0.12
Margaric (C17:0)	0.34 ± 0.02	0.34 ± 0.03	0.33 ± 0.01	0.34 ± 0.01
Stearic (C18:0)	11.26 ± 0.17	11.41 ± 0.22	11.45 ± 0.20	11.27 ± 0.28
Vaccenic (C18:1n9t)	4.41 ± 0.09	4.42 ± 0.06^{cd}	4.38 ± 0.05	4.43 ± 0.17
Oleic (C18:1n9c)	39.45 ± 1.63	40.65 ± 0.67^{a}	40.21 ± 1.53	40.44 ± 2.52
Linolic (C18:2n6c)	0.05 ± 0.00	0.05 ± 0.00	0.05 ± 0.00	0.05 ± 0.00
Linolenic (C18:3n3)	0.27 ± 0.02^{d}	0.28 ± 0.02^{d}	0.27 ± 0.04^{d}	0.26 ± 0.02
Eicosenoic (C20:1n^{-11})	0.50 ± 0.04	0.55 ± 0.04^{a}	0.52 ± 0.03^{a}	0.54 ± 0.04^{ac}
Eicosapentaenoic (C20:5n^{-3})	0.10 ± 0.01	0.10 ± 0.01	0.10 ± 0.01	0.11 ± 0.01
Arachidonic (C20:4n^{-6})	1.48 ± 0.22	1.52 ± 0.13	1.60 ± 0.15^{a}	1.60 ± 0.18^{a}
Docosapentaenic (C22:5n^{-6})	0.14 ± 0.00	0.14 ± 0.01	0.14 ± 0.01	0.15 ± 0.01
Docosahexaenic (C22:6n^{-3})	0.04 ± 0.00	0.045 ± 0.01	0.04 ± 0.01	0.04 ± 0.01
SFA	39.80 ± 1.23	39.93 ± 1.05	39.39 ± 0.87	39.36 ± 1.43
MUFA	51.36 ± 1.93	51.83 ± 1.86	52.56 ± 1.30^{d}	50.17 ± 3.51
PUFA	11.29 ± 1.42^{b}	10.94 ± 0.61	11.19 ± 0.32	11.37 ± 1.50^{bc}
PUFA/ SFA	0.28 ± 0.04	0.28 ± 0.02	0.28 ± 0.01	0.29 ± 0.01
ω3	0.60 ± 0.05	0.57 ± 0.09	0.56 ± 0.05	0.56 ± 0.09
ω6	10.33 ± 1.41^{b}	9.84 ± 0.48	10.40 ± 0.46^{b}	10.64 ± 1.57^{bc}
CLA	0.13 ± 0.02	0.13 ± 0.01	0.13 ± 0.01	0.14 ± 0.01

$^{a\,b\,c\,d}$ = values within the same different superscripts differ significantly ($p \leq 0.05$)

Table 4: The essential amino acid composition of MLD muscles (g.100.g^{-1})

Characteristics (n = 6)	Control-C	1EG - with $ZnSO_4.H_2O$	2EG - with Glycinoplex Zn	3EG - with Bioplex Zn
	$\bar{x}$ ± SD	$\bar{x}$ ± SD	$\bar{x}$ ± SD	$\bar{x}$ ± SD
Threonine	1.18 ± 0.05	1.15 ± 0.03	1.20 ± 0.08^{b}	1.16 ± 0.09
Valine	1.10 ± 0.04	1.10 ± 0.02	1.12 ± 0.07^{abd}	1.10 ± 0.08
Methionine	0.85 ± 0.02^{b}	0.82 ± 0.01	0.84 ± 0.05	0.83 ± 0.06
Cystine	0.37 ± 0.01	0.37 ± 0.01	0.37 ± 0.02	0.36 ± 0.02
Isoleucine	1.03 ± 0.04	1.02 ± 0.02	1.04 ± 0.07	1.02 ± 0.09
Leucine	2.11 ± 0.08	2.09 ± 0.06	2.14 ± 0.13^{bd}	2.08 ± 0.20
Phenylalanine	1.09 ± 0.03	1.07 ± 0.03	1.10 ± 0.07	1.07 ± 0.09
Histidine	1.26 ± 0.06	1.25 ± 0.06	1.27 ± 0.08	1.25 ± 0.14
Lysine	2.26 ± 0.08	2.03 ± 0.07	2.28 ± 0.15^{b}	2.23 ± 0.21
Arginine	1.68 ± 0.06	1.66 ± 0.05	1.70 ± 0.11	1.66 ± 0.16
Σ EAA	12.92 ± 0.48	12.80 ± 0.32	13.06 ± 0.83	12.77 ± 1.17

$^{a\,b\,c\,d}$ = values within the same different superscripts differ significantly ($p \leq 0.05$)

CONCLUSION

The growth rate in all groups was independent of zinc treatment. There was no statistically significant difference between the experimental and control groups in parameters of growth performance and the carcass yield.

A non-positive influence of zinc, dose of 38.46 g Glycinoplex-Zn and 66.67 g Bioplex-Zn per 100 kg of feed on animal health raises levels of cholesterol, water holding capacity and energy value, and lowers the value of pH, was noted in *longissimus dorsi* muscle compared to rabbits fed the control diet. Supplementation of Glycinoplex-Zn (100 mg of zinc) evoked a 25 % mortality rate with frequent *Pasteurella* infections to 8.3 % of the Bioplex-Zn (100 mg of zinc) as is shown in Table 2. These conditions were not observed in rabbits fed with other diets. The fatty acids content investigated in this study has proven statistically insignificant changes ($p \leq 0.05$). The highest content of lysine, leucine, valine, threonine and sum of essential amino acids was specified in group 2EG with supplementation of Glycinplex-Zn ($p \leq 0.05$). Results of this study suggest that lean rabbit meat could be a high quality protein source due to its well-balanced essential amino acids composition. Obtained results indicate to the prerequisite for inclusion of other additives in feed for breeding rabbits.

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