

EFFECT OF DIETARY ZINC SUPPLEMENTATION ON NUTRIENTS DIGESTIBILITY AND FERMENTATION CHARACTERISTICS OF CAECAL CONTENT IN PHYSIOLOGICAL EXPERIMENT WITH YOUNG RABBITS

Ľ. CHRASTINOVÁ¹*, K. ČOBANOVÁ², M. CHRENKOVÁ¹, M. POLÁČIKOVÁ¹, Z. FORMELOVÁ¹, A. LAUKOVÁ², Ľ. ONDRUŠKA¹, M. POGÁNY SIMONOVÁ², V. STROMPFOVÁ², Z. MLYNEKOVÁ¹, A. KALAFOVÁ³, Ľ. GREŠÁKOVÁ²

¹NPPC – Research Institute for Animal Production Nitra, Slovak Republic
²Institute of Animal Physiology Slovak Academy of Sciences, Košice, Slovak Republic
³Slovak University of Agriculture in Nitra, Slovak Republic

ABSTRACT

The effects of orally administered zinc from inorganic or organic sources on selected parameters of nutrient digestibility and caecal fermentation pattern in rabbits 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 three 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 acromelanic line (crossbreed Nitra Rabbit, Californian Rabbit, Big Light Silver) were used. The feed mixture was additionally administered as follows: in the 1st experimental group 1EG by a dose of 27.47 g ZnSO, H,O (zinc sulphate monohydrate), in the 2nd group (2EG) by a dose of 38.46 g Glycinoplex-Zn and in the 3rd group (3EG) a dose of 66.67 g Bioplex-Zn, each per 100 kg. Rabbits were fed with complete pelleted mixtures ad libitum and had free access to water via a nipple drinker. Dietary supplementation of rabbits with zinc was carried out to determine its effects on growth of live weight and consumption of feed per unit of live weight growth. Between 77-81 days of age, four rabbits from each group were selected for digestibility tests using the balance method. On the 91st day of age (6 weeks after all experimental procedures), 6 animals from each group were slaughtered, caecum and appendix were separated, and the caecal samples were collected for analysis; pH, VFA, ammonia-N and lactic acid were determined. We did not find any differences among experimental groups in the digestibility coefficients of starch, N-Free Extract, organic matter and Ca, P, Mg, and Cu obtained through the balance method (P > 0.05), compared with the control group or those fed with 100 Zn mg.kg⁻¹ supplemented diets. Increase in the supplemental Zn level to 100 mg.kg⁻¹ diet resulted in significant increase in digestibility coefficients of Na, K, Fe, Mn (P < 0.05) and Zn (P < 0.01) compared to the control group. No significant effect of the diet was detected on caecum in relative weights of its content, as well as on dry matter content. Feeding of rabbits with inorganic or organic zinc sources did not influence selected biochemical parameters in caecal fermentation, as well as had no negative effect on the rabbit growth performance.

Key words: rabbits; zinc; digestibility of nutrients; caecal fermentation pattern

INTRODUCTION

Zinc (Zn) has an important role in numerous biological processes. Zinc is an essential component of many enzymes (for the activity of over 300 enzymes), and it has both structural and catalytic functions in metalloenzymes (McCall *et al.*, 2000). One of the most

important functions of Zn is related to its antioxidant role and its participation in the antioxidant defence system. The mechanism, by which Zn exerts its antioxidant action, is not well defined. However, it has been suggested that Zn increases the synthesis of metallothionein, a cystine-rich protein that acts as

***Correspondence:** E-mail: chrastinova@vuzv.sk Ľubica Chrastinová, NPPC – Research Institute for Animal Production Nitra, Hlohovecká 2, 951 41 Lužianky, Slovak Rebublic Tel.: +421 37 6546 364 Received: November 26, 2015 Accepted: February 22, 2016 a free radical scavenger (Oteiza et al., 1996).

Zinc is absorbed in the small intestine and an intestinal pool of Zn may be formed by binding the metal to the intestinal metallothionein or Zn may be transported by albumin plasma to the liver (Prasad, 1993). More than one isoform of metallothionein is found in different tissues of animal species. Recently, a family of Zn transporters that play an important role in the regulation of Zn metabolism at the intracellular level in mammalians has been described. They structurally consist of 6 transmembrane domains, an intracellular histidine-rich region, and the amino and carboxy terminus, which resides intracellularly (Tako *et al.*, 2005).

On the other hand, a single isoform of metallothionein in the chicken has been found in liver, pancreas, kidney and intestinal mucosa (McCormick, 1984; Sandoval *et al.*, 1998). Metallothionein is synthesized in tissues in response to dietary Zn and can bind 7 atoms of Zn per molecule of protein, but they can also bind Cu with a higher affinity (Cousins and Lee-Ambrose, 1992).

Several biochemical and different clinical manifestations of Zn deficiency have been reported. Blood Zn concentrations are lower and the activity of several enzymes in metabolic pathways decreases in Zn-deficient animals. Zinc deficiency causes a loss of appetite and reduced efficiency of feed utilization and thus leads to growth retardation (Ensminger *et al.*, 1990; Mc Dowell, 2003).

Zinc deficiency in animals is characterized by decreased feed intake, decreased growth, low circulating levels of growth hormone (GH) and insulinlike growth factor-I, and decreased hepatic production of insulin-like growth factor-I, GH receptor and GH binding protein. Zinc positively affects feed utilization through participating in the metabolism of carbohydrates, lipids, and proteins (McDonald, 2000). 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 et al., 1988; Szentmihalyi et al., 1985; Haenlein, 1987).

Because of many natural food ingredients show marginal Zn-deficiency, this micronutrient is commonly supplemented to diets for livestock and poultry. Regardless of the fact that certain microelements are present in sufficient quantities in food, subclinical or clinical symptoms of their deficiency appear. This can be cause of their different and changeable availability, or the microelements are present in form that cannot be used. Obtained results showed that the presence of certain substances in food (phytic acid and oxalic acid), as well as interaction with other nutrients in the digestive tract, influencing resorption mechanisms. Resorption of microelements is not dependent only on their content in food, but also on the animals' age, electrochemical reactions in the intestine, and on the microelement form. Mineral salts are most frequently used, such as oxides, carbonates, chlorides and sulphates. Today, in addition to inorganic forms of minerals, the use of so-called "chelate" forms, i.e. organically bound microelements, is becoming more frequent.

The effects of orally administered zinc from inorganic or organic sources on selected parameters of nutrient digestibility and caecal fermentation pattern in rabbits were the priority of this study.

MATERIAL AND METHODS

Animals

A total of 96 rabbits (35th day of age, both sexes) were randomly divided into four groups (control C and three experimental groups - EG) with 24 animals in each group. The rabbits of meat line M91, maternal albinotic line (crossbreed New Zealand white, Buskat rabbit, French silver) and line P91, paternal acromelanic line (crossbreed Nitra's rabbit, Californian rabbit, Big light silver) were used in this experiment. Rabbits were housed in standard cages (0.61 m x 0.34 m x 0.33 m) with two animals per cage. The cages allowed the separation of faeces. The environmental temperature ranged from 24 °C to 31 °C, relative humidity of 65 %; these values were recorded continually using thermograph positioned at the same level as the cages. A cycle of 16 hours light and 8 hours of dark was used throughout the experiment. The animals were healthy and their condition was judged as good at the beginning of the experiment. In this animal study institutional and national guidelines for the care and use of animals were followed. Each experimental procedure, which involves animals, was approved by the State Veterinary and Food Institute of the Slovak Republic.

Experimental design

The animals were fed with complete pelleted feed (pellets of 3.5 mm in diameter) ad libitum and had free access to water via a nipple drinker. The diets were composed of 36 % dehydrated lucerne meal, 5.5 % extracted sunflower meal, 5.5 % extracted rapeseed meal, 9 % wheat bran, oats 13 %, malt sprouts 15 %, DDGS (dried distillers grains with soluble) 5 %, sodium chloride, mineral and vitamin mixture*, barley grains 8 %, limestone 1 %. The diet did not contain any anti-coccidial drug. The feed mixture was additionally administered:

in the 1st experimental group by dose of 27.47 g ZnSO₄. H_2O (Zinc sulphate monohydrate), in the 2nd group (2EG) by dose of 38.46 g of Glycinoplex-Zn and in the 3rd group (3 EG) dose of 66.67 g Bioplex Zinc, each per 100 kg. The rabbits in the group C were fed with the same commercially available diet with no zinc additive. The chemical composition of all feeds was determined by Weende (AOAC, 2000). The fattening experiment lasted for 48 days.

Dietary supplementation of rabbits with zinc was carried out to determine its effect on live weight growth and consumption of feed per unit of live weight growth. Rabbit's body weight and feed consumption were measured every week of the experiment. Mortality and morbidity were also recorded in the groups daily, over the entire period of the experiment. In the morning on 91st day of age (6 weeks after all experimental procedures) 6 animals from each group were electrically stunned and killed by cutting the carotids and jugular veins, then the carcasses were refrigerated for 24 h at 4 °C. Raw meat samples were packed and stored at -25 °C until they were analyzed. Caecum and appendix were separated and sampled for biochemical analyses.

Digestibility study

Total tract apparent digestibility was measured according to E.G.R.A.N. (2001), four rabbits (males, 2550 ± 100 g live body of weight) from each group were housed individually in metabolic cages (between 77 - 81 days of age). The adaptation period for this diet was 28 days. The faeces were collected individually during four consecutive days according to the European reference method for rabbit digestion trials (Perez et al., 1995). Sampling of faeces was performed every 2 hours. Faeces were collected in bags during the day time. Every day, in the morning, faeces were mixed with a handy mixer, the average samples were pre-dried (at 60 °C for 36 h in a dryer) and grinded (1 mm screen) with laboratory grinder for chemical analysis. Chemical analyses were conducted according to AOAC (2000) with the considerations mentioned by E.G.R.A.N. (2001) for dry mater (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 analyzed sequentially (Van Soest et al., 1991) with a thermostable amylase pre-treatment. Starch was determined by the enzymatic method according to Salomonsson et al. (1984). For macro- and microelements analysis the samples were ashed at 550 °C, the ash was dissolved in 10 ml of HCl (1:3) and minerals were determined by the atomic absorption spectrometry (AAS) method, phosphorus content was determined by molybdovanadate reagent on Camspec M501. Mineralized samples were analysed for Ca, P, Mg, Na, K, Fe, Zn, Cu and Mn content. For mineral

content determination the spectrometer AAS iCE 3000 (Thermo, UK) was used.

Contents of mineral nutrients in feeds and faeces were estimated in graphite cuvette through electrothermal atomization. Content of Ca was estimated at the wave length of 422.7 nm, Mg at 285.2 nm, Na at 589.0 nm, K at 766.5 nm, Fe at 248.3 nm, Zn at 213.9 nm, Cu at 324.8 nm, Mn at 279.5 nm and content of P at 410.0 nm as phosphomolybdenic yellow (Official Journal L 206, 29/07/1978, p.0043-0055). Each estimation was done in three replications. The nutrient digestibility was calculated according to following formula:

% D = (Intake – Faecal Excretion) / Intake x 100

Results were evaluated by statistical method such as significance of differences, the analysis of variance, one-way ANOVA and *t-test*, which were performed at P level less than 0.05.

Parameters in caecum

The caecal samples from each of three slaughtered rabbits (on the 91st day of age) were collected for biochemical analysis. VFA, pH, ammonia-N and lactic acid were determined; pH was measured immediately after sampling using a digital pH meter, VFA concentration was determined using gas chromatography on a 1.8 m glass column with 10 % SP1200 and 1 % H_3PO_4 on Chromosorbe WAW 80/100 mesh with isokaprylic acid as an internal standard (GC Carlo Erba). Ammonia-N concentration was measured by the micro diffusion according to Conway (Voigt and Steger, 1967). Lactic acid levels were determined by gas chromatography.

Statistical analysis

The statistical analysis was performed for all monitored traits. A linear model and a one-way analysis of variance were used for data analysis. Least square mean estimates with standard errors of the estimates were created. Differences among least square means were estimated and tested using the Tukey-test. The statistical package SAS 9.1 (SAS, 2003) was used for the analysis.

RESULTS AND DISCUSSION

The trial was carried out from July to August 2014 in the experimental house of the NAFC – Research Institute for Animal Production Nitra, Slovak Republic. The effects of dietary zinc supplementation with inorganic or organic substances on digestibility of nutrients and caecal fermentation pattern in rabbits were the priority of this study. Experimental animals did not show any health problems during the whole study period. Feeding was performed using balanced mixed feed according to feeding standards. The ability to discriminate among

diets varying in Zn concentration has been described for several animal species and nutrients. Zn is important for the organism and has influence on the feed intake; however, there is a lack of data whether rabbits can discriminate among diets differing in mineral content to avoid Zn-deficiency. The contribution of crude fibre is optimized up to the level of 14 - 16 % in rabbit mixture (NRC, 1980). Feeds were prepared at the beginning of the trial and stored at ambient temperature until they were provided to the rabbits. The samples of individual feeds were analyzed for content of nutrients, macro and micro elements (Table 1) according to AOAC (2000). Feed analyses were performed in triplicates. The content of digestible energy was calculated by the equation of Wiseman *et al.* (1992).

The chemical composition of feed gives an indication of the potential nutrient supply, but determination of digestibility provides an estimate of the nutrients available to the animal. Higher proportion of Zn in the mixture had influence on increase of in digestibility coefficients (P > 0.05) compared to control group. We did not find large differences among experimental groups in digestibility coefficients of starch, N-Free Extract, organic matter and Ca, P, Mg, and Cu using the balance method (P > 0.05) compared to the control or those fed with 100 Zn mg.kg⁻¹ supplemented diets. Pascual et al. (2008) recorded different coefficients of digestibility for dry matter, organic matter and gross energy (P < 0.05) between two different groups of rabbit does selected for litter size and longevity. Al-Dobain (2010) followed the effect of the diet on digestibility of four rabbit breeds. The author observed that all digestibility coefficients were significantly (P < 0.01) affected by the interaction dietary treatments × genetic groups. Increasing the supplemental Zn level to 100 mg.kg-1 diet resulted in significant increase

		С	1EG	2EG	3EG
Chemical analysis	Unit		– with	– with	– with
		Control- C	$ZnSO_4.H_2O$	Glycinoplex- Zn	Bioplex- Zn
Dry matter	g	903.78	896.00	901.13	894.31
Crude protein	g	176.23	173.09	175.09	174.52
Fat	g	35.81	37.68	37.96	37.91
Crude fibre	g	158.49	157.87	146.52	145.24
ADF	g	177.62	179.41	179.08	184.58
NDF	g	338.15	311.65	312.09	311.69
Starch	g	138.94	136.58	134.61	138.94
N-Free Extract	g	461.30	458.12	472.21	461.30
Hemicellulose	g	160.87	160.87	160.87	160.87
Cellulose	g	134.57	134.57	134.57	135.34
Ash	g	71.94	71.94	71.94	71.94
Organic matter)	g	831.84	831.84	831.77	831.84
Calcium (Ca)	g	7.68	7.63	7.58	7.28
Phosphorus (P)	g	6.94	6.96	6.95	7.01
Magnesium (Mg)	g	2.64	2.63	2.73	2.71
Sodium (Na)	g	1.64	1.64	1.67	1.43
Potassium (K)	g	11.75	10.78	11.06	11.06
Iron (Fe)	mg	342.98	415.22	373.04	457.27
Zinc (Zn)	mg	126.38	246.47	246.47	257.27
Copper (Cu)	mg	20.34	21.15	22.53	22.73
Manganese (Mn)	mg	164.67	164.67	164.67	164.67
ME	MJ.kg ⁻¹	11.07	10.94	11.37	11.43

Table 1: Content of nutrients and energy value in pelleted feed mixtures (kg per original matter)

*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.

of digestibility coefficients for Na, K, Fe, Mn (P < 0.05) and Zn (P < 0.01) compared to the control group. Abd El-Rahim *et al.* (1995) found that dietary supplementation of rabbit diet with 170 Zn mg.kg⁻¹ diet improved live body weight gain and feed conversion ratio. The digestibility of nutrients in different rabbit genotypes was studied by several authors (De Blas and Wiseman, 2010; Ondruška *et al.*, 2010).

Kustos and Hullár (1992) investigated the heritability of digestibility in NZW rabbits. In their experiment, the authors determined low ($h^2 = 0.19$) heritability values for the coefficients of digestibility. Lebas (1973; 1990) in the NZW breed determined 4 % better coefficients of digestibility for dry matter and organic matter than in the Californian rabbits; these coefficients of digestibility are in concert to our results. Rafay *et al.* (2009) and Maertens and Lebas (1989) specified these values of digestibility of basic nutrients: crude protein -75 %, crude fat -65 % and crude fibre -20 %.

Digestibility coefficients for crude protein and crude fibre in our experiment were higher than published by Tůmová *et al.* (2004), and Ondruška *et al.* (2011). These authors carried out a balance experiment on meat

rabbits and their digestibility values of presented nutrients were 77.2 % vs. 72.6 % (crude protein) and 10.7 % vs. 15.7 % (crude fibre). The effect of dietary zinc supplementation with inorganic or organic substances on the nutrient digestibility is presented in Table 2. The digestibility coefficients for crude protein were in the range from 77.72 % to 78.77 %, which was similar to the data of Battaglini and Grandi (1988). The values of crude fibre digestibility (15.23 % – 20.64 %) and crude fat (86.70 % – 89.26 %) were higher in comparison to those of Bielaňski and Niedžwiadek (1993). The values of zinc digestibility (3.35 % – 19.84 %) were lower in comparison to other herbivore species, e. g. goats.

Similar relationships between the minerals are also observed in Cu deficiency, but they are less pronounced, which means that the absorption of Cu increases with Zn deficiency but that the converse is not true (Memisi *et al.*, 2014). Different relationships between mineral absorption were observed with the goats received bentonite, which increased the absorption of Fe but has decreased absorption of Cu and Zn (Schwarz and Werner, 1987 Siegert *et al.*, 1986). Nessrin *et al.* (2012) studied response of growing rabbits to different supplemental

Item $(n = 4)$	Control (C)	1EC - with	2EC-with	3EC – with	t-test
		$ZnSO_4.H_2O$	Glycinoplex- Zn	Bioplex- Zn	
Dry matter	63.63 ± 1.82	63.12 ± 1.54	62.50 ± 1.43	63.14 ± 0.61	n.s.
Crude protein	78.50 ± 2.24	77.16 ± 0.67	78.02 ± 1.67	78.77 ± 1.04	n.s.
Fat	86.70 ± 1.61	89.26 ± 0.64	88.42 ± 2.84	89.20 ± 1.04	n.s.
Crude fibre	18.98 ± 2.81	20.64 ± 2.44	18.87 ± 2.99	15.24 ± 1.31	a:d+;b:d++
ADF	22.02 ± 2.24	19.70 ± 3.77	20.09 ± 4.36	25.06 ± 1.21	a:d+
NDF	35.33 ± 1.69	31.14 ± 2.22	39.61 ± 4.01	28.01 ± 2.80	a:b,c,d+
Starch	91.83 ± 0.90	94.75 ± 0.89	93.74 ± 1.34	93.50 ± 0.62	n.s.
N-Free Extract	73.31 ± 1.88	72.42 ± 1.71	71.51 ± 1.61	72.28 ± 0.74	n.s.
Hemicellulose	50.06 ± 1.41	46.66 ± 3.12	42.40 ± 5.35	34.91 ± 5.18	a:d++; b:d+
Cellulose	24.74 ± 2.46	21.50 ± 2.74	23.21 ± 5.27	27.13 ± 2.54	a:b+;b:d+
Ash	51.15 ± 1.75	49.10 ± 1.39	47.53 ± 0.91	48.35 ± 1.70	a:c+
Organic matter	64.02 ± 1.57	64.30 ± 1.15	63.75 ± 1.58	64.39 ± 0.59	n.s.
Calcium (Ca)	43.32 ± 5.59	53.89 ± 3.35	50.70 ± 4.14	49.02 ± 3.66	n.s.
Phosphorus (P)	32.06 ± 6.83	30.00 ± 1.80	30.15 ± 3.01	29.02 ± 3.01	n.s.
Magnesium(Mg)	25.57 ± 3.37	27.26 ± 0.69	26.30 ± 1.68	30.16 ± 3.01	n.s.
Sodium (Na)	86.29 ± 4.95	84.18 ± 4.23	70.53 ± 6.07	87.84 ± 9.91	a:c+
Potassium (K)	87.10 ± 3.73	86.82 ± 0.55	88.95 ± 0.84	89.45 ± 0.97	$b:d^+$
Iron (Fe)	37.50 ± 9.77	49.73 ± 0.77	40.88 ± 3.73	40.89 ± 3.73	$b:d^+$
Zinc (Zn)	3.35 ± 1.37	11.10 ± 2.07	19.84 ± 3.49	15.43 ± 1.72	a:c,d++
Copper (Cu)	17.74 ± 5.15	14.93 ± 7.17	13.00 ± 4.37	16.04 ± 5.05	n.s.
Manganese (Mn)	22.17 ± 4.27	24.85 ± 1.96	27.58 ± 2.38	32.51 ± 2.10	a:d+; b:d+
Control-C; 1EG – with ZnSO ₄ :H ₂ O; 2EG – with Glycinoplex-Zn; 3EG – with Bioplex-Zn; n.s. = $P > 0.05$; $^+ = P \le 0.05$; $^- = P \le 0.05$;					

Table 2: Coefficient of nutrient digestibility in % (Mean ± SD)

levels of zinc, magnesium or iron by following the growth performance and some carcass traits. Results of their work showed that Zn supplementation at levels of 100 or 200 mg.kg⁻¹ diet significantly (p < 0.05) improved live weight gain and feed conversion ratio compared to the higher level of the diet (400 mg.kg⁻¹).

Absorption of zinc occurs throughout the small intestine, usually in ranges from 5 % to 40 % for intake. Transfer of zinc out of the intestinal mucosal cells to the plasma is regulated by metallothionein. Zinc absorption is reduced whenever diets are high in calcium or phytate.

Studies worldwide have shown that in some countries, regarding the presence of certain minerals in the soil, there are different variations in terms of their deficit and surplus (Anke *et al.* 1988, 1993).

Ayyat and Marai (2000) reported that supplementing rabbits with 100, 200 or 300 Zn mg.kg⁻¹ significantly increased live weight gains, but had no effect on feed intake, feed conversion ratio or dressing percentage of the rabbits compared with the control or those fed 400 Zn mg.kg⁻¹ supplemented diets.

Generally we can conclude that the Zn concentrations (dose of 100 mg Zn.kg⁻¹ supplemented diets) used in this study have weak and/or do not have negative effect on feed intake, feed conversion ratio or dressing percentage of the rabbits compared with the control group (Table 4).

The rabbits were slaughtered before morning feeding for observation of fermentation processes in the caecum. There were no significant differences in pH value between control and experimental groups. Concentration of observed VFA shows, that the most intensive process was in the caecum of rabbits in the control group. The lower concentration of ammonia- N affects pH value in the control and experimental groups (Hoover and Heitmann, 1975). There were no significant differences (P > 0.05) between

Table 3: Qualitative parameters in caecum

		1EG – with	2EG - with	3EG – with
Parameters	Control (C)	ZnSO ₄ .H ₂ O	Glycinoplex- Zn	Bioplex- Zn
(n = 6)	$\overline{x} \pm SD$	$\overline{x}\pm SD$	$\overline{x}\pm SD$	$\overline{x}\pm SD$
pН	5.85 ± 0.32	6.21 ± 0.18	6.07 ± 0.36	6.09 ± 0.12
N-NH3 (mmol.1 ⁻¹)	14.36 ± 7.587	13.04 ± 3.30	12.93 ± 5.35	12.55 ± 3.56
Acetate (mmol.100 g ⁻¹)	7.039 ± 0.925	5.844 ± 0.651	6.853 ± 1.247	6.358 ± 1.322
Propionate (mmol.100 g ⁻¹)	0.569 ± 0.211	0.619 ± 0.076	0.550 ± 0.134	0.479 ± 0.036
Butyrate (mmol.100 g ⁻¹)	2.260 ± 1.380	1.541 ± 0.876	2.060 ± 1.293	1.589 ± 0.872
Other VFA (mmol.100 g ⁻¹)	0.513 ± 0.388	0.298 ± 0.129	0.284 ± 0.161	0.230 ± 0.132
Lactic acid (g.100 g ⁻¹)	0.0094 ± 0.003	0.0096 ± 0.001	0.0088 ± 0.003	0.0071 ± 0.005

n.s. = P > 0.05

Table 4: Growth performance of rabbits in response to dietary supplementation with zinc from inorganic or organic sources ($\bar{x} \pm SD$)

Parameters $(n = 24)$	Control (C)	1EG - with $ZnSO_4$ ·H ₂ O	2EG – with Glycinoplex- Zn	3EG – with Bioplex- Zn
Initial weight in g	1637 ± 119	1633 ± 33	1663 ± 183	1638 ± 93
Final weight in g	2971 ± 160	3004 ± 229	3049 ± 207	2954 ± 189
Daily weight gain in g.day ⁻¹	31.76	32.63	33.00	31.34
Feed conversion ratio in g.g ⁻¹	4.23	4.08	4.20	4.26
Carcass yield in %	59.24 ± 0.78	59.41 ±1.59	60.12 ± 0.45	58.37 ± 3.38

n.s. = P > 0.05

the observed rabbits, as well as between the control group and the treatments (Table 3). High lactic acid concentration was in the caecum of rabbits with the supplemented $ZnSO_4.H_2O$. These results are in agreement with those observed by many authors (Yoshida *et al.*, 1972; Hossain and Bertechini, 1993; Colina *et al.*, 2001). Crude fibre is digested by a microbial fermentation and main place of this fermentation is caecum (Volek *et al.*, 2005). No significant effect of the diet was detected on both caecum relative weights of its content, as well as on dry matter content. Before experimental period the animals were found in good health conditions.

CONCLUSION

The current experiment was conducted to evaluate the effect of two zinc products (Glycinoplex-Zn and Bioplex-Zn), supplemented to diet, on digestibility of basic nutrients and on fermentation processes in the caecum of broiler rabbits. This study demonstrated that rabbits are able to distinguish between diets differing in Zn-content and that Zn- deficiency causes a possibly learned preference for Zn. However, the ability to avoid Zn-deficiency by feed selection seems to be influenced by several factors. However, it could be concluded that growing rabbit is tolerable to excessive dietary doses of the macroelements or Zn.

Also, a supplemental Zn in the rate of 100 mg.kg⁻¹ diet leads to improving live body weight gain and significantly improves feed conversion ratio of the rabbit. Additionally, the environmental impact of zinc can be reduced.

ACKNOWLEDGEMENT

The work was supported by the APVV project (grant APVV-0667-12 "Zinc in animal nutrition and consumer safety"). The authors would like also to thank Dr. R. Jurčík and Dr. M. Ryzner, for help with the rabbit treatment and excellent technical assistance in the experiments.

REFERENCES

ABD El-RAHIM, M. I. A. – EL-GAAFARY, M. N. – TAWFEEK, M. I. – EL-KELAWY, H. M. – RAWIA. S. A. 1995. Effect of dietary supplementation with different levels of zinc on growth performance, nutrient digestibility, mineral metabolism, blood constituents, organ histopathology and reproductive efficiency in NZW rabbits. *Egyptian Journal of Rabbit*

- AL-DOBAIB, S. N. 2010. Effect of diets on growth, digestibility, carcass and meat quality characteristics of four rabbit breeds. *Saudi Journal of Biological Sciences*, vol. 17 (1), 2010, p. 83–93.
- ANKE, M. GROPPEL, B. KRAUSE, U. ANGELOW,
 L. REGIUS, A. MASAOKA, T. KOSLA, T. –
 LANGER, M. 1988. Diagnostic possibilities of the status of zinc, manganese, copper, iodine, selenium, molybdenum, cadmium, nickel, lithium and arsenic.
 In: *Proc. Macro- and Trace Element Seminar*, University Leipzig-Jena, Germany, Dec. 20-21, 1988, p. 368.
- ANKE, M. MEISSNER, D. MILLS, C. F. 1993. Trace Elements in Man and Animals - Tema 8. *Proceedings* of the 8th International Symposium on Trace Elements in Man and Animals, 1993, 1156 p.
- AOAC (2000). Official Methods of Analysis of AOAC International, 17th ed. (Ed. Horwitz, W.), AOAC International, Gaithersburg, USA.
- AYYAT, M. S. MARAI, I. F. 2000. Growth performance and carcass traits as affected by breed and dietary supplementation with different zinc levels, under Egyptian condition. In: 7th World rabbit Congress. July 4-7, 2000, Spain, p. 83–88.
- BATTAGLINI, M. GRANDI, A. 1988. Trifolium pratense L. Hay in diets of growing rabbits. In: *Proc.* 4th World Rabbit Congress, 1988, p. 123–131.
- BIELAŇSKI, P. NIEDŽWIADEK, S. 1993. The use of rapeseed "00" in complete mixtures for rabbits. In: 8th Symposium on Housing and Diseases of Rabbits, Furbearing Animals and Fancy Pet Animals in Celle, 1993, p. 135-140.
- COLINA, J. J. LEWIS, A. J. MILLER, P. S. – FISCHER, R. L. 2001. Dietary manipulation to reduce aerial ammonia concentration in nursery pig facilities. *Journal of Animal Science*, vol. 79, 2001, p. 3096–3103.
- COUSINS, R. J. LEE-AMBROSE, L. M. 1992. Nuclear zinc uptake and interactions and metallothionein gene expression are influenced by dietary zinc in rats. *Journal of Nutrition*, vol. 122, 1992, p. 56–64.
- De BLAS, C. WISEMAN, J. 2010. The nutrition of the Rabbit. 2nd ed. Cabi, www. CABI org., 2010, 325 p. ISBN 978-1-84593-669-3.
- E.G.R.A.N. 2001. Technical note: Attempts to harmonize chemical analyses of feeds and faeces for rabbit feed evaluation. *World Rabbit Science*, vol. 9, 2001, p. 57–64.
- ENSMINGER, M. E. OLDFIELD, J. E. HEINEMANN, W. W. 1990. Feeds and nutrition. In: The Eisminger Publ. Comp. Clovis, CA. 1990, p. 8–120.
- HAENLEIN, G. F. W. 1987. Mineral and vitamin requirements and deficiencies. In: Proc. IVth International Conference Goats, Brasilia, Brazil,

March 8-13, 1987, p. 1249.

- HOOVER, W. H. HEITMANN, R. N. 1975. Caecal nitrogen metabolism and amino acid absorption in the rabbit. *Journal of Nutrition*, vol. 105, 1975, p. 245–252.
- HOSSAIN, S. BERTECHINI, A. G. 1993. Requirement of zinc for growing rabbits. Arquivo Brasileriro. *Medicina Veterinaria Zootecnia*, 45, 1993, p. 323–329.
- KUSTOS, K. HULLÁR, I. 1992. Heritability of digestibility of feed dry matter and crude protein and their relationship with various production traits in New Zealand White rabbits. *Journal of Applied Rabbit Research*, vol. 15, 1992, p. 255–258.
- LEBAS, F. 1973. Variation, chez le lapin, des coefficients dutilisation digestive de la matiere skche, de la matiere organique et del'azote en fonction de l'fige de la race et du sexe. *Annales de Biologie Animale, Biochimie, Biophysique*, vol. 13, 1973, p. 365.
- LEBAS, F. 1990. Recherche et alimentation des lapines. *Cunculture*, vol. 17, 1990, p. 13–15.
- MAERTENS, L. LEBAS, F. 1989. Energy evaluation of rabbit feeds and feedstuffs. A critical approach. *Cuni-Sciences*, vol. 5, 1989, p. 35–46.
- McCALL, K. A. HUANG, C. FIERKE, C. A. 2000. Function and mechanism of zinc metalloenzymes. *Journal of Nutrition*, vol. 130, 2000, p. 1437–1446.
- Mc DONALD, R. S. 2000. The role of zinc in growth and cell proliferation. *Journal of Nutrition*, vol. 130, (5S Suppl.), 2000, p. 1500–1508.
- Mc CORMICK, C. C. 1984. Induction and accumulation of metallothionein in liver and pancreas of chicks given oral zinc: A tissue comparison. *Journal of Nutrition*, vol. 114, 1984, p. 191–203.
- Mc DOWELL, L. R. 2003. Minerals in Animal and Human Nutrition, 2nd ed. Elsevier Science B. V., 2003, Amsterdam, The Netherlands.
- MEMIŠI, N. LEVIĆ, J. ILIĆ, N. 2014. The influence of presence of zinc in diet on production traits of goats. In: *Proc. XVI Int. Symposium "Feed Technology"* 28-30 October, 2014, Novi Sad, Serbia, p. 78-87, ISBN 978-86-7994-044-5.
- NATIONAL RESEARCH COUNCIL 1980. Mineral tolerance of domestic animals. National Research Council. National Academy Press, Washington, D.C., vol. 3, 1980, p. 41–43.
- NESSRIN, S. ABDEL KHALEK, A. M. GAD, S. M. 2012. Effect of supplemental zinc, magnesium or iron on performance and some physiological traits of growing rabbits. *Asian Journal of Poultry Science*, vol. 6 (1), 2012, p. 23–30.
- ONDRUŠKA, L. CHRASTINOVÁ, Ľ. CHRENEK, P. – RAFAY, J. – PARKÁNYI. V. 2010. Digestibility of nutrients by transgenic and non-transgenic rabbits. *Slovak Journal of Animal Science*, vol. 43 (4), 2010, p. 210–214.

- ONDRUŠKA, Ľ. CHRASTINOVÁ, Ľ. RAFAY, J. – POSPÍŠILOVÁ, D. – PARKÁNYI, V. 2011. Vplyv humínových látok a probiotík na rast a produkčné ukazovatele brojlerových králikov. In: Nové směry v intenzivních a zájmových chovech králíků - XI. Celostátní seminář, 16. 11. 2011, Praha, Česká republika, s. 35–39. ISBN 978-80-7403-083-3.
- OTEIZA, P. L. OLIN, K. L. FRAGA, C. G. KEEN, C. L. 1996. Oxidant defence systems in testes from Zn deficient rats. *Proceedings of the Society for Experimental Biology and Medicine*, vol. 213, 1996, p. 85–91.
- PEREZ, J. M. LEBAS, F. GIDENNE, T. MAERTENS,
 L. XICCATO, G. PARIGI-BINI, R. DALLA
 ZOTTE, A. COSSU, M. E. CARAZZOLO. A.
 VILLAMIDE, M. J. CARABANO, R. FRAGA,
 M. J. RAMOS, M. A. CERVERA, C. BLAS,
 E. FERNANDE Z-CARMONA, J. FALCAOE
 CUNHA, L. BENGALA FREI RE, J. 1995.
 European reference method for *in vivo* determination
 of diet digestibility in rabbits. *World Rabbit Science*,
 vol. 3, 1995, p. 41–43.
- PASCUAL, J. J. BASELGA, M. BLAS, E. RÓDENAS, L. – CATALÁ, A. E. 2008. Differences in digestive efficiency between rabbit does selected for litter size at weaning and for reproductive longevity. In: *Proc. of the 9th World Rabbit Congress*, Verona, 10-13 June, 2008, p. 205–209.
- PRASAD, A, S. 1993. Biochemistry of Zinc. Plenum Press, New Nork, NY.
- RAFAY, J. SŰVEGOVÁ, K. CHRASTINOVÁ, Ľ. PARKANYI, V. ONDRUŠKA, Ľ. CHRENEK, P. 2009. Chov králikov. Publikácia CVŽV Nitra 20, 2009, 137 p. ISBN 978-80-89418-00-8.
- SAS Release 9.1 SAS Institute Inc. Cary, USA, 2002-2003.
- SALOMONSSON, A. C. THEANDER, O. WESTERLUND, O. 1984. Chemical characterization of some Swedish cereal whole meal and brain fraction. Swedish. *Journal of Agricultural Research*, vol. 14, 1984, p. 111–117.
- SANDOVAL, M. HENRY, P. R. LUO, X. G. LITTELL, R. C. – MILES, R. D. – AMMERMAN, C. B. 1998. Performance and tissue zinc and metallothionein accumulation in chicks fed a high dietary level of zinc. *Poultry Science*, vol. 77, 1998, p. 1354–1363.
- SCHWARZ, T. WERNER, E. 1987. The effect of long term bentonite application upon the metabolism of selected trace elements in dwarf goats. In: *Proc. Macro- and Trace Element Seminar*, University Leipzig-Jena, Germany, Dec. 21-22, 1987.
- SIEGERT, E. ANKE, M. SZENTMIHALYI, S. REGIUS, A. – LOKAY, D. – POWEL, J. – GRUEN, M. 1986. The zinc supply of plants and animals

in Middle Europe. In: Proc. 5th Intern. Trace Element Symposium, University Leipzig-Jena, Germany, July 14–17, 1986, 487 p.

- SZENTMIHALYI, S. SIEGERT, E. HENNIG, A. – ANKE, M. – GROPPEL, B. 1985. Zinc contents of flora in relation to age, geology of soil and plant species. In: *Proc. Macro- and Trace Element Seminar*, University Leipzig-Jena, Germany, Dec. 2-3, 1985, p. 466.
- TAKO, E. FERKET, P. R. UNI, Z. 2005. Changes in chicken intestinal zinc exporter mRNA expression and small intestinal functionality following intra - amniotic zinc - methionine administration. *Journal of Nutritional Biochemistry*, vol. 16, 2005, p. 339–346.
- TŮMOVÁ, E. SKŘIVANOVÁ, V. ZITA, L. SKŘIVAN, M. – FUČÍKOVÁ, A. 2004. The effect of restriction on digestibility of nutrients, organ growth and blood picture in broiler rabbits. In: 8th World rabbit Congress. 2004, p. 1008–1014. [CD-ROM].
- VAN SOET, P. J. ROBERTSON, J. B. LEWIS, B. A. 1991. Methods for dietary fiber, neutral detergent fiber, and starch polysaccharides in relation to animal nutrition. *Journal of Dairy Science*, vol. 74, 1991, p. 3583–3597.

- VOIGT, J. STEEGER, H. 1967. Zur quantitativen Bestimmung von Ammoniak, Harnstoff und Ketokörpern in biologischem Material mit Hilfe eines modifizierten Mikrodiffusionsgefässes. Archiv für Tierernaehrung, vol. 17, 1967, p. 289–293.
- VOLEK, Z. MAROUNEK, M. SKŘIVANOVÁ, V. 2005. Replacing starch by pectin and inulin in diet of early – weaned rabbits: effect on performance, health and nutrient digestibility. *Journal of Animal and Feed Sciences*, vol. 14, 2005, p. 327–337.
- WISEMAN, J. VILLAMIDE, M. J. De BLAS, C. – CARABANO, M. J. – CARABANO, R. M. 1992. Prediction of the digestible energy and digestibility of gross energy of feed for rabbits. 1. Individual classes of feeds. *Animal Feed Science and Technology*, vol. 39, 1992, p. 27–38.
- YOSHIDA, T. PLEASANTS, J. R. REDDY, B. S. – WOSTMANN, B. S. 1972. The pH values and nitrogen fractionations of cecal contents and feces of germfree and conventional rabbits. *Japanese Journal of Zootechnical Science*, vol. 43, 1972, p. 284–289.