## EFFECT OF COMBINATIVE DIETARY ZINC SUPPLEMENTATION AND PLANT THYME EXTRACT ON GROWTH PERFORMANCE AND NUTRIENT DIGESTIBILITY IN THE DIET FOR GROWING RABBITS

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## ABSTRACT

The aim of this study was to evaluate the effect of combinative orally administered zinc from organic source (Bioplex-Zn) and 0.1 % of *Thymus vulgaris* L. plant extract to feed mixtures for rabbits, applied into water during the growing period, on the selected parameters of nutrient digestibility. Feeding trial was conducted on 96 post-weaned rabbits, meat line P91 and M91 (aged 35 days, both sexes). They were randomly divided into 4 experimental groups (EG) with 24 animals in each group. The rabbits in the EG1 and EG3 were fed the same commercially available diet with no zinc additive. The feed mixture in the EG2 and EG4 was additionally administered at a dose of 33.3 g Bioplex-Zn per 100 kg each (this is addition of 50 mg Zn.kg<sup>-1</sup> feed mixture). The rabbits in the group EG3 and EG4 received 0.1 % of *Thymus vulgaris* L. plant extract applied into the water. Supplementation of the rabbit diets with zinc was done in order to determine its effects on live weight and consumption of feed per unit of live weight growth. Higher proportion of Zn in the mixture increased digestibility coefficients of fat, NDF, starch, organic matter, Mg, Na, K, Fe, Zn, Cu, Mn (P < 0.05) compared to EG1 with no zinc additive. Natural product of *Thymus vulgaris* L. extract consisted of flavonoids (54 %), diterpene (27 %) and phenolic acids (19 %), which are known to have an antimicrobial and antioxidant activity. The beneficial effect of combination of the *Thymus vulgaris* L. plant extract and zinc additive administration was manifested by the anti-coccidian action in rabbits. These results indicate that the addition of 50 mg Zn.kg<sup>-1</sup> of a mixture is sufficient to achieve optimal health, performance, increased feed conversion rate (p < 0.05) and average daily weight gain in broiler rabbits.

Key words: rabbits; Bioplex-zinc; plant extract; nutrient digestibility

## INTRODUCTION

Minerals have a special role in ensuring efficient growth, reproduction and immunocompetence in animals. The content of trace elements in the feed is governed by the nature of geochemical soil and plant species (Suttle, 2010). Zinc deficiency in animals is characterized by decreased feed intake, decreased growth (Ensminger *et al.*, 1990), low circulating levels of growth hormone (GH) and insulin-like 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 carbohydtrates, lipids and proteins (McDonald, 2000). Minerals activate enzymes which are essential cofactors of metabolic reactions and function as carriers of proteins, regulate digestion,

\*Correspondence: Email: chrenkova@vuzv.sk Mária Chrenková, NPPC – Research Institute for Animal Production Nitra, Hlohovecká 2, 951 41 Lužianky, Slovak Republic Tel.: +421 37 6546 217 respiration, water balance, muscle response and 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.*, 1993; Szentmihalyi *et al.*, 1985; Haenlein, 1987; Underwood and Suttle, 1999).

Because 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 food in sufficient quantities, subclinical or clinical symptoms of their deficiency appear. This can be caused by 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, influences 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, such as oxides, carbonates, chlorides and sulphates are most frequently used. Today, in addition to inorganic forms of minerals, the use of so-called "chelate" forms, i.e. organically bonded microelements, is becoming more frequent. Enteric diseases frequently occur in rabbits around the weaning period, leading to an extensive use of antibiotics. Therefore, new and safe antimicrobial agents are searched for to prevent and/or overcome infections. Prevention and treatment of clostridial infections by natural substances or phyto-additives are important because of animal mortality and economic losses (Chrastinová et al., 2010; Chrastinová et al., 2016; Marcin et al., 2006). Natural products of plant origin (in this case the extract of Thymus vulgaris L.) are still a major part of traditional medicine. However, their effect on animal and human organisms is still not clear. Essential oils have recently emerged as alternatives to antibiotics in animal production (Simonová et al., 2006; Takáčová et al., 2012; Szabóova et al., 2008).

The aim of this study was to examine the effects of combinative orally administered zinc from organic source. The effect of Bioplex-Zn and

*Thymus vulgaris* L. plant extract at 0.1 % applied into drinking water on growth performance and selected parameters of nutrient digestibility in rabbits was the main goal of this study.

## MATERIAL AND METHODS

A total of 96 weaned rabbits ( $35^{th}$  day of age, both sexes, meat line P91 and M91) were divided into 4 experimental groups (EG) with 24 animals in each group. The rabbits were kept in standard cages ( $0.61 \text{ m} \times 0.34 \text{ m} \times 0.33 \text{ m}$ ), two animals per cage. The cages allowed faeces separation. A cycle of 16 h of light and 8 h of dark was used throughout the experiment. Temperature and humidity were recorded continuously by a digital thermograph positioned at the same level as the cages. Heating and forced ventilation systems allowed air temperature in the building to be maintained within  $24 \pm 4$  °C during the experiment. Relative humidity was  $70 \pm 5$  %.

## **Experimental procedures**

The rabbits in the EG1 and EG3 groups were fed the same commercially available diet without zinc additive. The animals were fed complete pelleted feed (pellets of 3.5 mm in diameter) *ad libitum* and had free access to water.

The experimental diets were balanced for nutrient content and energy value (difference was in the content of zinc, Table 1). The contents of organic matter were not different. The study was carried out at the National Agricultural and Food Centre – Research Institute for Animal Production, Nitra, Slovak Republic in July and August 2017.

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 8 %, limestone 1 %. To the feed mixture in the EG2 and EG4, Bioplex-Zn (product of Alltech, USA) was additionally administered at a dose of 33.3 g per each 100 kg (this is addition of 50 mg Zn.kg<sup>-1</sup> into feed mixture). The rabbits in EG3 and EG4 groups received *Thymus vulgaris* L. (0.1 % of plant extract) applied into drinking water. Natural products of plant origin (in this case the extract of *Thymus vulgaris* L.) are still a major

Nutrients in dry matter (g.kg <sup>-1</sup> )	Basal diet	Basal diet supplemented with Bioplex Zinc	
Crude protein	172.54	172.55	
Crude fibre	166.73	169.07	
Fat	41.10	41.17	
N-Free Extract	536.68	534.62	
Starch	183.29	182.19	
Organic matter	917.05	917.41	
ADF	209.46	214.13	
NDF	334.36	346.04	
Hemicellulose	124.90	131.91	
Cellulose	169.75	167.22	
Ash	82.95	82.59	
Calcium	6.52	6.64	
Phosphorus	6.34	6.53	
Iron, mg. kg <sup>-1</sup>	507.30	517.49	
Zinc mg. kg <sup>-1</sup>	106.56	161.53	
Copper, mg. kg <sup>-1</sup>	18.24	19.05	
Manganese, mg. kg <sup>-1</sup>	133.66	125.38	
ME (MJ.kg <sup>-1</sup> )	10.99	10.84	

## Table 1. Chemical composition of the experimental diets for growing rabbits

Vitamin mixture provided per kg of the diet: Vit. A 1500000 IU; Vit. D<sub>3</sub> 125000 IU; Vit. E 5000 mg; Vit. B<sub>1</sub> 100 mg; Vit. B<sub>2</sub> 500 mg; Vit. B<sub>6</sub> 200 mg; Vit. B<sub>12</sub> 0.01 mg; Vit. K3 0.5 mg; biotin 10 mg; folic acid 25 mg; nicotinic acid 4000 mg; choline chloride 100000 mg; ADF - Acidodetergent fibre; NDF - Neutraldetergent fibre; ME - Metabolisable energy

part of traditional medicine. The extract of *Thymus vulgaris* L. contains especially flavonoids (54 %), diterpene (27 %) and phenolic acids (19 %), which are known to be antimicrobials and antioxidants.

All care and experimental procedures involving animals kept the guidelines stated in the Guide for the Care and Use of Laboratory Animals (1996), and the trials were accepted by the Ethical Commission at the Institute of Animal Physiology in Košice and by the Slovak Veterinary and Food Administration.

Body weight and feed consumption were measured every week during the experiment. Mortality and morbidity were also recorded in the groups daily, over the entire period of the experiment. The fattening experiment lasted for 42 days.

#### In vivo digestibility trial

The digestibility test using the balance method was measured according to E.G.R.A.N. (2001). This method was developed within the European group on Rabbit Nutrition. Between 70-74 days

of age, 4 rabbits (males, 2250 ± 100 g live body weight) from each group were housed individually in metabolic cages. The adaptation period for this diet was 28 days. The faeces were collected individually during 4 consecutive days according to the European reference method for rabbit digestion trials (Perez et al., 1995). Sampling of faeces was realized every 2 hours. Faeces were collected in bags during the daytime. Every day, in the morning, faeces were mixed with a handheld 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) 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 polarimetric method on Polarimeter ADP 220 (Bellingham & Stanley Ltd., UK). For macro- and microelement analysis, 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 determined 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). All the analyses were performed in triplicates. The nutrient digestibility was calculated according to the following formula:

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

#### The occurrence of *Eimeria* sp. oocysts in the faeces

*Eimeria* sp. oocysts were enumerated in the faecal samples microscopically at the start of the experiment (day 0-1) and on the 42<sup>nd</sup> day of the experiment and expressed in counts of oocysts per 1 g of faeces (OPG). The samples were evaluated by the quantitative flotation technique - modified McMaster method (Ministry of Agriculture, Fisheries and Food, Manual of veterinary parasitological laboratory techniques, 1986).

Data were statistically evaluated using the SAS/STAT 1999-2001 statistical software (version 82). The results were quoted as the mean  $\pm$  standard deviation (SD); statistical evaluation of the results was performed by a one-way ANOVA and Tukey's test. The significance level was set at p < 0.05.

## **RESULTS AND DISCUSSION**

The chemical composition of feeds (Table 1) gave an indication of the potential nutrient supply, but determination of digestibility provides an estimate of the nutrients available to the animal. 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. Enteric diseases frequently occur in rabbits around the weaning period, leading to an extensive use of antibiotics. Therefore, new and safe antimicrobial agents are searched for to prevent and / or overcome infections. Prevention and treatment of clostridial infections by natural substances or phyto-additives are important because of animal mortality and economic losses. The duration of the fattening experiment was 42 days. Body weight and feed consumption of rabbits were measured every week during the experiment. Among the experimental groups significant differences in feed intake, body weight and carcass value in fattening experiment (Table 2). Dietary supplementation of zinc to rabbits was carried out to determine its effects on live weight gain and consumption of feed per unit of live weight gain. The average daily weight gain was higher in all experimental groups: EG2 (40.91 g), EG3 (41.05 g), EG4 (41.51 g) comparing to the EG1 (basal diet without zinc additive - 38.55 g). Coccidiosis in rabbit breeds presents a serious health and economic problem (Vasilková et al., 2007; Pakandl, 2009). Between 7 and 8 weeks of age, mortality was registered in the group EG3, and only 2 rabbits were died by diarrhoea at 49 and 56 d of age. The overall mortality at the end of growing-fattening phase was 1 (EG1) vs. 2 rabbits (EG3), what could be caused by intake of the basal diet without zinc additive (Bioplex-Zn) and higher intake of starch in the diet (18.3 %) and 0.1 % concentration of thyme plant extract applied into water. Basal diet with supplementary Bioplex-Zn combined with 0.1 % thyme plant extract in water in a preventive way to improve intestinal health and reduce the need for non-sustainable practices. However, the general consensus of opinion is that overload of rapidly fermentable carbohydrates in the large intestine increases the likelihood of digestive disorders, at least in susceptible, recently weaned rabbits due to problems with enterotoxemia induced by Clostridia (Cheeke, 1987; De Blas et al., 1995; De Blas and Gidenne, 1998).

Parameter (n = 24)	EG1	EG2	EG3	EG4
Initial weight (g)	1161 ± 119	1114 ± 136	1071 ± 124	1114 ± 149
Final weight (g)	2779 ± 300	2830 ± 268	2796 ± 208	2859 ± 344
Daily weight gain g.day <sup>-1</sup>	38.55 ± 6.61	40.91 ± 7.50	41.05 ± 4.05	41.52 ± 5.55
Feed intake g.day <sup>-1</sup>	134	124 <sup>A</sup>	131	134
Feed conversion ratio in g.g <sup>-1</sup>	3.51	3.04 <sup>A</sup>	3.22ª	3.23ª
Contrast to the basal diet (%)		+13.39 <sup>A</sup>	+9.17ª	+7.98ª
Mortality (n)	1	0	2	0
Carcass yield (%)	55.65 ± 0.71	55.88 ± 2.23	54.92 ± 0.46	57.17 ± 0.71

## Table 2. Effects of feed supplementation with zinc (additive Bioplex-Zn) and combinative administration with 0.1 % plant extract *Thymus vulgaris* on performance data of the fattening rabbits (mean ± SD)

EG1 - Basal diet without supplementary Zinc; EG2- Basal diet with supplementary with Bioplex- Zn at ; EG3- Basal diet without supplementary Zinc and with thyme plant extract 0.1 % in water; EG4- Basal diet with supplementary Bioplex-Zn combined with thyme plant extract 0.1 % in water;  $a = p \le 0.05$ ; h = Significant difference at  $p \le 0.01$ 

The chemical composition of feeds give an indication of the potential nutrient supply, but determination of digestibility provides an estimate of the nutrients available to the animal. The data of nutrient digestibility are summarized in Table 3.

Higher proportion of Zn in the mixture had influence on the increase of fat, NDF, starch, organic matter, Mg, Na, K, Fe, Zn, Cu, Mn digestibility coefficients (P < 0.05) compared with EG1 with no zinc additive. Lebas (1973; 1990) in the NZW rabbit breed determined 4 % better coefficients of digestibility for dry matter and organic matter than in the Californian rabbits; these coefficients of digestibility correspond to our results. Several authors (Rafay et al., 2009; Maertens, 1992; Lebas, 1989) specified these values of digestibility of basic nutrients as follows: crude protein - 75 %, crude fat - 65% and crude fibre - 20%. Digestibility coefficients of 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 a dose of 33.3 g Bioplex-Zn (organic substances) per each 100 kg on nutrient digestibility is presented in Table 3. The resulting digestibility coefficients for crude protein were in the range from 77.93 % to 82.48 %, which was similar to the data of Battaglini and Grandi (1988). The values of crude fibre digestibility (23.48 % - 29.60 %) and crude fat (83.92 % - 90.32 %) were higher in comparison to Bielaňski and Need Świadek (1993).

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). Minerals have a special role in ensuring efficient growth and immunocompetence in animals. The content of trace elements in the feed is governed by the nature of geochemical soil and plant species. Addition of 3 % microalgae spirulina (Arthrospira platensis) and/or 2.5 % thyme (Thymus vulgaris) leaves in growing (7 weeks) dwarf rabbits did not prevent the animals from getting sick or dying during the 14 weeks of the study period, and there were no substantial effects on the growth performance and energy or nutrient digestibility (Dalle Zotte et al. 2013). Absorption of zinc occurs throughout the small intestine and usually in ranges from 5 % to 40 % for intake. Zinc absorption is reduced whenever diets are high in calcium or phytate. The values of zinc digestibility (1.52 % - 16.96 %) 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, what means that the absorption of Cu increases with Zn deficiency, but that the converse is not true (Memiši et al., 2014).

Different relationships between mineral absorption were observed with the goats that received bentonite, which increased the absorption

Item (n=4)	EG1	EG2	EG3	EG4	t-test
Crude protein	78.24 ± 1.04	82.48 ± 1.50	78.71 ± 0.84	77.93 ± 1.83	a:b <sup>+</sup> ;b:c <sup>++</sup> , d <sup>+</sup>
Fat	83.92 ± 2.55	89.75 ± 0.64	84.82 ± 2.23	90.32 ± 0.69	a:bd <sup>+</sup> ; c:d <sup>++</sup>
Crude fibre	25.81 ± 0.62	29.60 ± 2.51	23.48 ± 0.81	25.77 ± 3.83	a:bc <sup>+</sup> ; b:c <sup>+</sup>
ADF	29.42 ± 3.43	37.74 ± 4.36	28.55 ± 1.88	28.71 ± 0.83	a:bc+; b:c+d+
NDF	34.40 ± 2.65	41.10 ± 3.91	36.23 ± 3.57	34.82 ± 1.35	a:b⁺, c, d
Starch	93.97 ± 0.93	94.83 ± 0.96	96.84 ± 0.17	97.09 ± 0.08	a:cd <sup>++</sup> ; b:cd <sup>+</sup>
N-Free Extract	73.31 ± 1.88	79.41 ± 1.64	75.01 ± 1.10	74.41 ± 1.59	b:cd⁺
Hemicellulose	39.16 ± 5.62	43.45 ± 9.39	49.79 ± 7.15	31.78 ± 4.09	a:d++; b:d+
Cellulose	32.41 ± 3.01	43.98 ± 9.07	24.15 ± 7.22	28.33 ± 1.17	a:bd⁺; b:cd⁺
Ash	52.75 ± 2.93	62.43 ± 1.16	51.96 ± 1.08	51.83 ± 2.13	b:a <sup>+</sup> c <sup>++</sup> d <sup>+</sup>
Organic matter	65.90 ± 0.36	70.79 ± 1.80	65.76 ± 0.75	66.23 ± 1.00	b:acd⁺
Calcium (Ca)	59.41 ± 3.70	65.63 ± 4.75	44.20 ± 6.73	47.48 ± 3.66	a:bcd <sup>+</sup> ; b:cd <sup>+</sup>
Phosphorus (P)	40.48 ± 6.83	49.81 ± 5.75	37.40 ± 4.82	39.05 ± 2.08	a:b⁺; b:acd⁺
Magnesium (Mg)	47.37 ± 6.38	53.42 ± 4.64	46.31 ± 3.17	50.83 ± 3.38	b:c⁺
Sodium (Na)	80.60 ± 5.02	84.18 ± 4.23	70.66 ± 13.49	82.42 ± 2.59	b:d⁺
Potassium (K)	84.15 ± 2.12	87.13 ± 1.27	85.29 ± 0.84	87.07 ± 4.18	a:bd <sup>+</sup> ; b:c <sup>+</sup>
Iron (Fe)	26.09 ± 4.12	37.18 ± 2.51	35.05 ± 3.81	25.93 ± 3.01	a:bc <sup>+</sup> ; c:d <sup>++</sup>
Zinc (Zn)	$1.52 \pm 0.86$	2.87 ± 2.04	4.23 ± 2.13	16.96 ± 2.28	a:c <sup>+</sup> , d <sup>++</sup>
Copper (Cu)	13.88 ± 4.11	31.84 ± 4.98	18.48 ± 2.65	20.69 ± 6.87	a:b <sup>+</sup> ; b:cd <sup>+</sup>
Manganese (Mn)	19.80 ± 2.06	21.23 ± 1.50	20.02 ± 1.64	19.55 ± 1.29	b:c, d⁺

Table 3. Effects of feed supplementation with zinc (additive Bioplex-Zn) and combinative administration
with 0.1 % plant extract <i>Thymus vulgaris</i> on nutrient digestibility in % (mean ± SD)

 $^{\scriptscriptstyle +}$  = p  $\leq$  0.05;  $^{\scriptscriptstyle ++}$  = p  $\leq$  0.01 Significant difference

of Fe but has decreased absorption of Cu and Zn (Schwarz and Werner, 1987; Siegert et al., 1986; Stefanidou et al., 2006). However, by addition of thyme plant extract at the concentration of 0.1 %, a significant decrease of digestibility coefficients of ADF, cellulose, Ca, P, Mg ( $p \le 0.05$ ) was recorded in EG3 compared to values in the EG1. It appears that the antagonistic effect of thyme plant also appeared without zinc additive in mixture at digestibility of crude fibre when a significant decrease in the EG3 (23.48 %) was recorded compared to the EG1 (25.81%). The key trace elements involved in animal feed are zinc (Zn), copper (Cu), iron (Fe) and manganese (Mn) (Andrieu et al., 2009). Zinc is the second most abundant trace element in mammals and birds, and forms a structural component of over 300 enzymes, where it may also be key to catalytic and regulatory activity. It plays and important role in anti-oxidant defence as an integral part of SOD. Manganese plays an important role in the body metabolism as an essential part of several enzymes. These elements

have both negative and positive relationships within intermediary metabolism. Positive chemical bonds (Mn - Zn) are lesser than the antagonistic bonds (Cu - Zn). Absorption of Zn and Mn is inhibited also by Ca (Shajhalal *et al.*, 2008; Wilde, 2006). Copper is a component of a several range of physiologically important metalloenzymes, lipid metabolism, antioxidant defence as an integral part of the essential enzyme - superoxide dismutase (SOD), immune function and carbohydrate metabolism (Wapnir, 1998). Occurrence of secondary Zn deficiency in animals with excessive content of Ca, P and phytic acid in feed rations is frequent. Deficiency of Mn in soil and plants is due to excessive liming of the soil.

The anti-coccidian effect was recorded in experimental groups EG2, EG3 and EG4 on the 42<sup>nd</sup> day compared to EG1 (Table 4). In EG1 290 OPG were counted, whereas in EG2 20 OPG were found, and in the EG3 - 40 OPG. The beneficial effect of combination of the *Thymus vulgaris* L. plant extract and zinc additive administration manifested as the anti-coccidian effect in rabbits. By improving

# Table 4. Comparison of the counts *Eimeria* sp. Oocysts in faeces of rabbits (expressed in counts of oocysts per 1 g (OPG) of faeces evaluated by the quantitative flotation technique)

Characteristic (n=5)	EG1	EG2	EG3	EG4
OPG	290	20**	40**	Neg.**

EG1- Basal diet without supplementary Zinc; In the EG2 and EG4 the feed mixture was additionally administered as follows a dose of 33.3 g Bioplex-Zn, each per 100 kg. The rabbits in the group EG3 and EG4 received dose 0.1 % plant extract *Thymus vulgaris* were applied into supply water.  $^{++}P < 0.01$ 

intestinal health, possibly through stimulation of the processes of nonspecific immunity, this feed additive may result in a lower exposure of animals to microbial toxins or other undesired metabolites (Plachá *et al.*, 2013). Mr. Ján Pecho, Mr. Igor Matušica, Ing. Ľubomír Ondruška, Dr. Rastislav Jurčik and Dr. Vladimir Parkányi for their excellent technical assistance and co-operation in the experiments.

## CONCLUSION

The experimental results show that dietary supplementation of zinc to rabbits was carried out to determine its effects on growth of live weight and consumption of feed per unit of growth weight. No significant differences were observed in mean percentage of the carcass gain in tested variants. The beneficial effect of combination of the Thymus vulgaris plant extract and zinc additive administration was manifested by the anticoccidian action in rabbits. Higher proportion of Zn in the mixture had influence on the increase of digestibility coefficients of fat, NDF, starch, organic matter, Mg, Na, K, Fe, Zn, Cu and Mn (P < 0.05) compared to EG1 with no zinc additive. On the basis of obtained results we can conclude that the total addition of 50 mg Zn per kg of mixture is sufficient to achieve optimal health and performance of the rabbits and increased feed conversion rate and average daily weight gain (p < 0.05). Consequently, this additive can enhance immune defence in critical situations, increase the intestinal availability of essential nutrients for absorption, and improve the growth of animals.

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## REFERENCES

- ANDRIEU, M.S. FAROOQ, A.A. MUSHTAQ, M. 2009. Serum concentrations of coper, iron, zinc and selenium, in cyclic and anoestrus Nili-Ravi buffaloes kept under conditions. *Pakistan Veterinary Journal*, vol. 29, 2009, p. 47–48.
- 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: *Proceedings*of Macro- and Trace Element Seminar, University
  Leipzig-Jena, Germany, 1988, December 20-21, p. 368.
- ANKE, M. MEISSNER, D. MILLS, C.F. 1993. Trace Elements in Man and Animals – Tema 8. In: Proceedings of the 8<sup>th</sup> International Symposium on Trace Elements in Man and Animals, 1993, 1156 p.
- AOAC 2000. Official Methods of Analysis, 17<sup>th</sup> ed., Association of Official Analytical Chemists, Gaithersburg, MD, USA.
- BATTAGLINI, M. GRANDI , A. 1988. Trifolium pratense
   L. Hay in diets of growing rabbits. In: Proceedings of the 4<sup>th</sup> World Rabbit Congress, 1988, p. 123–131.
- BIELAŇSKI, I. NEED ŚWIADEK, S. 1993. The use of rapeseed "00" in complete mixtures for rabbits. In: 8<sup>th</sup> Symposium on Housing and Diseases of Rabbits, Furbearing Animals and Fancy Pet Animals in Celle, 1993, p. 135–140.
- CHEEKE, P.R. 1987. *Rabbit Feeding and Nutrition*. Academic Press Inc. Orlando.
- CHRASTINOVÁ, Ľ. ČOBANOVÁ, K. CHRENKOVÁ, M. – POLÁČIKOVÁ, M. – FORMELOVÁ, Z. – LAUKOVÁ,

A. – ONDRUŠKA, Ľ. – POGÁNY SIMONOVÁ, M. – STROMPFOVÁ, V. – MLYNEKOVÁ, Z. KALAFOVÁ, A. – GREŠÁKOVÁ, Ľ. 2016. Effect of dietary zinc supplementation on nutrients digestibility and fermentation characteristics of caecal content in physiological experiment with young rabbits. *Slovak Journal of Animal Science*, vol. 49, 2016 (1), p. 23–31.

- CHRASTINOVÁ, Ľ. CHRENKOVÁ, M. LAUKOVÁ, A. – POLÁČIKOVÁ, M. – SIMONOVÁ, M. – SZABÓOVÁ, R. – STROMPFOVÁ, V. – ONDRUŠKA, Ľ. – CHLEBEC, I. – PARKÁNYI, V. – RAFAY, J. – VASILKOVÁ, Z. 2010. Influence of selected phytoadditives and probiotics on zootechnical performance, caecal parameters and meet quality of rabbits. *Archiva Zootecnia*, vol. 13, 2010, p. 30–35.
- DALLE ZOTTE, A. SARTORI, A. BOHATIR, P. REMIGNON, H. – RICCI, R. 2013. Effect of dietary supplementation of Spirulina (*Arthrospira platensis*) and Thyme (*Thymus vulgaris*) on growth performance, apparent digestibility and health status of companion dwarf rabbits. *Livestock Science*, vol. 152, 2013, p. 182–191.
- De BLAS, J.C. TABOADA, E. MATEOS, G.G. NICODEMUS, N. – MENDEZ, J. 1995. Effect of substitution of starch for fibre and fat in isoenergetic diets on nutrient digestibility and reproductive of rabbits. *Journal of Animal Science*, vol. 73, 1995, p. 1131–1137.
- De BLAS, E. GIDENNE, T. 1998. Digestion of starch and sugars. *The Nutrition of the Rabbit*. (de BLAS C. and WISEMAN, J. eds., CABI Publishing, 1998, p. 17–38.
- EGRAN. European group on Rabbit Nutrition. 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.* The Eisminger Publ. Comp. Clovis, CA. 1990, p. 8–120.
- HAENLEIN, G.F.W. 1987. Mineral and vitamin requirements and deficiencies. In: *Proceedings of the IV<sup>th</sup> International Conference on Goats*, Brasilia, Brazil, 1987. March 8-13, p. 1249.
- 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. 1989. Besoins nutritionnels des lapins. Revue bibliographique et perspectives. *Cuni-Sciences*, 5 (2), 1989, p. 1–28.

- LEBAS, F. 1990. Recherche et alimentation des lapines. *Cuniculture*, vol. 17, 1990, p. 13–15.
- MAERTENS, L. 1992. Rabbit nutrition and feeding: a review of some recent developments. In: Cheeke, P.R. (ed.) Proceedings of the 5<sup>th</sup> World Rabbit Congress. Oregon State University, Corvallis, Oregon, USA, p. 889–913.
- MARCIN, A. SÚSTRIKOVÁ, A. MATI, R. 2006: The effects of aromatic oils on growth performance and physiological parameters in the intestine of weaned pigs. *Slovak Journal of Animal Science*, vol. 39, 2006, p. 103–107.
- MEMIŠI, N. LEVIĆ, J. ILIĆ, N. 2014. The influence of presence of zinc in diet on production traits of goats.
  In: Proceedings of XVI International Symposium "Feed Technology", 28-30.10.2014, Novi Sad, Serbia, p. 78–87, ISBN 978-86-7994-044-5.
- 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.
- Ministry of Agriculture, Fisheries and Food 1986: *Manual* of veterinary parasitological laboratory techniques, 3<sup>rd</sup> Ed. London: Her Majesty's Stationery Office (HMSO), 1986, 160 p.
- 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, p. 35–39, ISBN 978-80-7403-083-3.
- PAKANDL, M. 2009. Coccidia of rabbit: a review. *Folia Parasitologica*, vol. 56 (3), 2009, p. 153–166.
- PEREZ, J.M. LEBAS, F. GIDENNE, T. MAERTENS, L.
   XICCATO, G. PARIGI-BINI, R. DALLE YOTTE, A. –
  COSSU, M.E. CARAZZOLO, A. VILLAMIMIDE, M.J.
   CARABAŇO, R. FRAGA, M.J. RAMOS, M.A. –
  CERVERA, C. BLAS, E. FERMANDEZ CARMONA, J.
   FALCAO, E. CUNHA, L. BENGALA FREIRE, J. 1995.
  European reference method for *in vivo* determination
  of diet digestibility in rabbits. *World Rabbit Science*,
  vol. 3, 1995, p. 41–43.
- PLACHÁ, I. CHRASTINOVÁ, Ľ. LAUKOVÁ, A. ČOBANOVÁ, K. – TAKÁČOVÁ, J. – STROMPFOVÁ, V. – CHRENKOVÁ, M. – FORMELOVÁ, Z. – FAIX, Š. 2013. Effect of thyme oil on small intestine integrity and antioxidant status, phagocytic activity and gastrointestinal microbiota in rabbits. Acta Veterinaria Hungarica, vol. 61 (2), 2013, p. 197–208.

- RAFAY, J. SÜVEGOVÁ, K. CHRASTINOVÁ, Ľ. PARKÁNYI,
  V. ONDRUŠKA, Ľ. CHRENEK. P. 2009. *Chov králikov,*2. preprac. vyd. Nitra : CVŽV Nitra, 2009, 144 p.
  ISBN 978-80-89418-00-8.
- SCHWARZ, T. WERNER, E. 1987. The effect of long term bentonite application upon the metabolism of selected trace elements in dwarf goats. In: *Proceedings of Macro- and Trace Element Seminar*, University Leipzig-Jena, Germany, 1987, December 21-22, p. 99.
- SHAHJALAL, M. KHALEDUZZAMAN, A.B.M. KHANDAKER, Z.H. 2008. Micro mineral profile of cattle in four selected areas of Mymensingh district. *Bangladesh Journal of Animal Science*, vol. 37, 2008, p. 44–52.
- 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: *Proceedings of the 5<sup>th</sup> Interntional Trace Element Symposium*, University Leipzig-Jena, Germany, 1986, July 14-17, p. 487.
- SIMONOVÁ, M. STROMPFOVÁ, V. MARCIŇÁKOVÁ, M. – HAVIAROVÁ, M. – FAIX, Š. – VASILKOVÁ, Z. – LAUKOVÁ, A. – ŠALAMON, I. 2006: The experimental application of chamomile essential oil in rabbits. In: *Proceedings of the 1<sup>st</sup> International Symposium on Chamomile Research, Development and Production*; 2006, June 7-10, Prešov, Slovakia, 122 p.
- STEFANIDOU, M. MARAVELIAS, C. DONA, A. SPILIOPOULOU, C. 2006. Zinc: a multipurpose trace element. Archives of Toxicology, vol. 80, 2006, p. 1–9.
- TAKÁČOVÁ, J. RYZNER, M. PLACHÁ, I. ČOBANOVÁ, K. – FAIXOVÁ, Z. – FAIX, Š. 2012. *Thymus vulgaris* essential oil in poultry nutrition. In: *Book of Abstracts from* 25<sup>th</sup> Days of Animal Physiology, 2012, October 17-19, Košice, Slovakia, p. 72, ISBN 978-80-968618-8-0.
- 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: 8<sup>th</sup> World Rabbit Congress. 2004, p. 1008–1014. [CD-ROM].

- SUTTLE, N.F. 2010. *The Mineral Nutrition in Livestock*, 4<sup>th</sup> ed., British Library, London, UK, 2010, p. 5–113.
- 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: *Proceedings of Macro- and Trace Element Seminar*, University Leipzig-Jena, Germany, 1985, December 2-3, p. 466.
- SZABÓOVÁ, R. LAUKOVÁ, A. CHRASTINOVÁ, Ľ.– SIMONOVÁ, M. – STROMPFOVÁ, V. – HAVIAROVÁ, M. – PLACHÁ, I. – FAIX, Š. – VASILKOVÁ, Z. – CHRENKOVÁ, M. – RAFAY, J. 2008. Experimental application of sage in rabbit husbandry. *Acta Veterinaria Brno*, vol. 77, 2008, p. 581–588.
- UNDERWOOD, E.J. SUTTLE, N.F. 1999. *The mineral Nutrition of Livestok*, 3<sup>rd</sup> ed., CABI Publishing. CAB International, Wallingford, UK.
- VAN SOEST, P.J. ROBERTSON, J.B. LEWIS, B.A. 1991. Methods for dietary fiber, neutral detergent fiber, and non-starch polysaccharides in relation to animal nutrition. *Journal of Dairy Science*, vol. 74, 1991, p. 3583–3597.
- VASILKOVÁ, Z. LAUKOVÁ, A. SZABÓOVÁ, R. SIMONOVÁ, M. – CHRASTINOVÁ, Ľ. – STROMPFOVÁ, V. – RAFAY, J. – ONDRUŠKA, Ľ. – PORAČOVÁ, J. 2007.
  Prírodné aditíva v chove králikov a ich vplyv na redukciu oocysts *Eimeria* spp. In: *Nové směry v chovu brojlerovych králíků*: Sborník referátů IX. celostátního semináře, 2007. Praha, Česká republika, p. 28–30, ISBN 978-80-86454-87-0.
- WAPNIR, R.A. 1998. Copper absorption and bioavailability. The American Journal of Clinical Nutrition, vol. 67, 1998, p. 1054–1060.
- WILDE, D. 2006. Influence of macro and micro minerals in the peri-parturient period on fertility in dairy cattle. *Animal Reproduction Science*, vol. 96, 2006, p. 240–249.