Acta Veterinaria (Beograd), Vol. 54. No. 2-3, 175-189, 2004.

UDK 619:636.4.084.415

CALCIUM, PHOSPHORUS, ZINC AND THEIR RATIOS IN SERUM OF FATTENING SWINE FED DIFFERENT DIETS IN RESPECT TO ZINC

RUPIĆ V*, LUTEROTTI SVJETLANA**, ČEPELAK IVANA**, REKIĆ BRANKICA***, GRBEŠA D* and KNEŽEVIĆ M*

**Faculty of Agriculture, University of Zagreb, Croatia*
***Faculty of Pharmacy and Biochemistry, University of Zagreb, Croatia*
****Medical Biochemical Laboratory Health Center, Zagreb Center, Croatia*

(Received 7. January 2004)

The influence of zinc supplementation in diets which contained constant concentrations of calcium and phosphorus, on serum concentrations of calcium, phosphorus and zinc and their molar ratios was investigated in fattening German Landrace x Piétrain x Large White x Swedish Landrace weaned crossbreds. Bioavailability of zinc from Zn-methionate was found to be significantly better than that of zinc from $ZnSO_4$ in the period of fast growth and body mass gain. The characteristic time profile for Zn in serum was observed in all experimental groups but at different zinc levels.

No marked influence of dietary supplemented zinc from either organic or inorganic source on serum Ca and P concentrations was recorded. Moreover, a consistent trend of P/Ca values following supplementation of zinc was not found. The supplemented zinc from both organic or inorganic source led to lasting decreases of P/Zn and Ca/Zn values. Therefore these ratios may be used as reliable indicators of Zn status in swine: P/Zn ≤300 and Ca/Zn ≤200 would indicate physiological concentrations of zinc in serum of pigs. Contrary to this the appearance of parakeratosis might be expected at low levels of zinc and relatively high Ca/Zn and P/Zn ratios.

Key words: pigs, nutrition, Zn, Ca, P, ratios

INTRODUCTION

Minerals constitute a small percentage of the swine diet but their importance to the health and well-being of the pig cannot be over-emphasised. Functions of minerals are diverse, ranging from structural functions in some tissues to a wide variety of regulatory functions. The appropriate supplementation of animals with minerals from sources of high bioavailability is one of the ways of keeping them in good health and physiological body growth.

Phosphorus is one of the essential structural components of cells and organelles; it is also involved in the generation, storage and release of metabolic energy (Endres and Rude, 2001). The essential portion of intracellular

phosphorus is present in the form of organic compounds, *e.g.* lipid and carbohydrate intermediary products and plays a role in fat, protein and carbohydrate metabolism, as well as in transport-related processes and cell growth. The kidneys are the regulators of phosphate homeostasis.

The skeleton is a major reservoir used to provide calcium for both the extracellular and intracellular pools (Endres and Rude, 2001). Extracellular calcium provides calcium ion for the maintenance of intracellular calcium, bone mineralisation, blood coagulation and plasma membrane potential. Calcium is also important in muscle contraction and as an intracellular second messenger affecting enzyme activity and hormone secretion.

Many investigations have been devoted to the transport of calcium and phosphorus and to their biological importance in animal health. Disturbances in transport of calcium and phosphorus cause skeletal diseases and disturbances in the function of the neuromuscular system known as osteodystrophy, tetany and hypocalcemic neurosis. In the case of nutritional deficiency bones serve as a reservoir of calcium and phosphorus for blood and tissues.

Zinc is an oligoelement which is an integral part of about 300 enzymes (*e.g.* carboanhydrase, alkaline phosphatase, alcohol dehydrogenase, RNA and DNA polymerases, 5-nucleotidase) which take part in the synthesis of proteins, metabolism of carbohydrates and nucleic acids (Milne, 1999). Transport of zinc and its biological importance for animals (cell replication, CO_2 release from lungs, sexual maturation, wound healing, fertility and reproduction) have been the topic of many investigations.

Concentrations of calcium, phosphorus and zinc and their ratios are very important for various physiological metabolic processes taking place in animals. Some investigations have revealed that quantitative disbalances of these elements may lead to pathological processes. The health of fattening pigs is of prime interest to all swine meat producers. Zinc is often added to swine nursery diets because it helps to maintain growth rates and performance. Tucker and Salmon (1955) showed that dietary Zn deficiency caused a skin disorder of fattening pigs known as parakeratosis**.** Calcium interferes with zinc absorption and therefore increased quantities of calcium in the diet may result in parakeratosis in pigs as well (Luecke *et al.*, 1957, Sanstead, 1994). However, parakeratosis may appear of even with diets low in Ca. According to the same authors the pathological effect of high amounts of dietary calcium on the metabolism of minerals in swine could be prevented by addition of 50 mg Zn/kg to the meal.

There are discrepancies between reports in the literature on the bioavailability of zinc from inorganic and organic sources. While Kirchgessner and Hartel (1977) found the highest bioavailability of zinc from a complex with an amino acid and the lowest from inorganic salts and Hill *et al.* (1986) described better productivity in pigs supplemented with organic zinc than inorganic zinc, Wedekind *et al.* (1994) did not observe such differences. Our own results (Rupić *et al.*, 1997) confirmed that zinc from complexes with amino acids, *e.g.* methionine, increases the productivity of fattening pigs. Pond *et al.* (1995) stated that the skin is rich in zinc and therefore sensitive to Zn deficiency and bioavailability from the

diet. Deficiency of dietary zinc affects the nuclei of epithelial skin cells leading to their complete degeneration; it also reduces and even stops the synthesis of nucleic acids and collagen. Zn deficiency significantly decreases the incorporation of some amino acids in to the proteins of the skin.

In this investigation we were interested in serum concentrations of calcium, phosphorus and zinc and in their ratios in particular, during the fattening of swine fed on basal diets with no extra supplementation of zinc and the effect of zinc supplements from both inorganic and organic sources.

MATERIALS AND METHODS

Animals and diets. A total of 42 pigs, German Landrace x Piétrain x Large White x Swedish Landrace weaned crossbreds, were divided in to three groups, T_1-T_3, with 14 pigs in each. The pigs were kept in boxes, 7 pigs per box (a total of 6 boxes). There were 7 males and 7 females in each group. All groups in the trial contained pigs from 7 litters born on the same day. An equal number of pigs from one litter (one male and one female) were included in each group. The state of the health pigs was checked daily.

The male piglets were castrated at the age of 14 days. All of them were marked with numbers, from 1 to 42, tattooed on the right ear.

The animal room had forced air circulation. The animals were kept under the same microclimatic conditions: the average air temperature was 18.0 ^oC and relative humidity 79%.

Pigs were offered the diets and water *ad libitum* by means of automatic feeding and watering systems. The composition of the diets is presented in Table 1. They provided all nutrients as recommended by the National Research Council (1998) except for Zn in the basal diet.

In the pre-trial period lasting for 30 days the pigs were acclimatised and fed the starter diet containing19.31% proteins and 45.50 mg/kg Zn. Afterwards all pigs were weighed and randomly divided into three trial groups T_1, T_2 and T_3. The groups were uniform in respect to number, sex and body mass. All groups received the same basic diet (Table 1) which changed with the animal body mass category. The trial lasted for 105 days. During first 28 days the pigs were fed the starteration (up to body mass of 30 kg), followed by the grower diet for another 28 days (up to body mass of 60 kg) and then during the last 49 days (up to the end of the trial) the finisher ration (on day 105 average body mass in groups T_2 and T_3 was 95 kg).

All trial groups received the required allowance of Ca and P in the diet: on average 0.70% calcium, namely 0.78% in the starter, 0.72% in the grower and 0.60% in the finisher and on average 0.52% phosphorus, namely 0.65% in the starter, 0.53% in the grower and 0.38% in the finisher.

The basal group (T_1) received diets with no extra zinc supplement during the whole trial. They contained on average 38.17 mg Zn/kg mix (45.50 in the starter, 38.00 in the grower, 31.00 in the finisher). Group T_2 received on average a total of 122.49 mg Zn/kg (128.00 in the starter, 124.26 in the grower and 115.20 in the finisher). Group T_3 received on average a total of 81.48 mg Zn/kg (101.80 in the

Table 1. Composition of diets fed to pigs

Ingredients (% as fed)	Starter (15-30 kg)	Grower (30-60 kg)	Finisher (60-105 kg)
Corn	46.05	42.50	39.50
Barley meal	22.0	33.45	41.00
Wheat middlings	–	–	3.50
Soybean meal	19.00	13.00	9.50
Sunflower meal	–	6.00	–
Alfalfa meal	1.50		3.40
Fodder yeast (vet.)	6.67		1.00
Fat	1.20	2.50	–
Iodized salt	0.30	–	0.50
Limestone	1.15	1.00	0.80
Dicalcium phosphate	1.00	0.70	0.30
DL-methionine	0.13	–	–
Vitamin-mineral premix without antibiotics SP*	1.00	–	–
Vitamin-mineral premix without antibiotics ST*	–	0.50	0.50
Total	100.00	100.00	100.00
Analysis as fed**			
Dry matter (%)	88.56	89.20	88.80
Crude protein (%)	19.31	15.68	13.30
Crude fat (%)	3.75	3.00	2.60
Crude fibre (%)	3.77	4.81	4.02
Ca (%)	0.78	0.72	0.60
P (%)	0.65	0.53	0.38
Mn (mg/kg)	46.00	48.70	52.37
Fe (mg/kg)***	315.00	178.26	139.40
Cu (mg/kg)	32.50	20.24	12.30
Zn (mg/kg) (T_1, basal)***	45.50	38.00	31.00
Zn (mg/kg) (T_2, $ZnSO_4$)†	128.00	124.26	115.20
Zn (mg/kg) (T_3, ZnMET)‡	101.80	80.30	62.33

* Vitamin-mineral premixes contain:
Zn-free VMP - SP provided the following per kilogram: vitamin A, 1,000.000 IU; vitamin D_3, 100,000 IU; vitamin E, 1,500 mg; vitamin K_3, 200 mg; vitamin B_1, 200 mg; vitamin B_2, 400 mg; niacin, 2,500 mg; *D*-pantothenic acid, 1,500 mg; vitamin B_6, 300 mg; vitamin B_{12}, 2 mg; biotin, 10 mg; choline chloride, 50,000 mg; Fe, 10,000 mg; Cu, 2,000 mg; Mn, 3,000 mg; I, 75 mg; Co, 30 mg; Se, 10 mg. Additions of Zn were 8,000 mg from $ZnSO_4$ and 4,000 mg from ZnMET.

Zn-free VMP - ST provided the following per kilogram: vitamin A, 1,000.000 IU; vitamin D_3, 100,000 IU; vitamin E, 2,400 mg; vitamin K_3, 400 mg; vitamin B_1, 400 mg; vitamin B_2, 600 mg; niacin, 3,000 mg; *D*-pantothenic acid, 2,000 mg; vitamin B_6, 400 mg; vitamin B_{12}, 3 mg; choline chloride, 100,000 mg; Fe, 16,000 mg; Cu, 4,000 mg; Mn, 8,000 mg; I, 150 mg; Co, 40 mg; Se, 20 mg. Additions of Zn were 16,000 mg from $ZnSO_4$ and 8,000 mg from ZnMET.
** Official methods were used throughout (AOAC, 1984).
*** Fe:Zn: 6.9 in starter, 4.7 in grower, 4.5 in finisher.
† After addition of $ZnSO_4$ to vitamin-mineral premix.
‡ After addition of ZnMET to vitamin-mineral premix.

starter, 80.30 in the grower, 62.33 in the finisher). Thus, groups T_2 and T_3 were supplemented with extra zinc: group T_2 with an average of 84.32 mg Zn/kg as $ZnSO_4$ (82.50 in the starter, 86.26 in the grower and 84.20 in the finisher), and group T_3 with an average of 43.31 mg Zn/kg in the form of Zn-methionate (56.30 in the starter, 42.30 in the grower and 31.33 in the finisher).

The source of inorganic zinc was $ZnSO_4xH_2O$ (B.B.V. Chemie, Duisburg, Germany) and of organic zinc it was Zn-methionate (PLIVA, Zagreb, Croatia). Zinc compounds were mixed with the vitamin-mineral premix.

Sampling and analyses. Deionized, distilled water was used throughout. All reagents used were of analytical grade. Disposable plasticware was used whenever possible.

Precautions were taken to avoid contamination with zinc. All glassware was acid-washed (soaked in 1+1 HNO_3 for 24 h and rinsed thoroughly with water). Blood was taken using special purpose trace element (low Zn) evacuated tubes with stainless steel needles. Blood samples were allowed to clot in Falcon polystyrene labware tubes (Falcon, Oxnard, CA, USA) and the serum was stored in the same type of tubes. Falcon tubes were used also for preparing sample dilutions.

Blood samples were taken from the brachial region *(v. cava cranialis*) at the same time of a day (08.00 to 11.00 h a.m.) from the same animals and in the same group sequence. Blood was sampled from each individual in the group after a 12-h fast. Some samples were lost during manipulations and storage.

Calcium and phosphorus in serum were determined spectrophotometrically on an multichannel auto analyser (Technicon RA-1000, Technicon Instruments Co., New York, NY, USA), based on the reaction of Ca with *o*-cresolphthalein complexone (Connerty and Briggs, 1966) and of P with molybden acid sulphate (Fiske and Subbarow, 1925).

Flame atomic-absorption spectrometry was used for determination of Zn in blood serum. Standard operating conditions were applied (Perkin-Elmer 305B atomic absorption spectrophotometer, Perkin-Elmer, Norfolk, CAT, USA). Samples were prepared by diluting sera 1+4 with water. Glycerol (5%, V/V) served as a blank and as the diluent for preparing the standard solutions (Butrimovitz and Purdy, 1977; Smith *et al.*, 1979). Lyophilised control serum Validate-A (Lot No. 101553, Organon Teknika, Eppelheim, Germany) was used to check the accuracy of the method.

Statistics. Statistical significance of differences between mean values was evaluated using Student's *t*-test. Outliers were identified by the *Q*-test and deleted.

RESULTS

Feeding variables. All experimental groups were uniform in respect to the starting body mass. The average starting body mass was 15.5 kg (T_1: 15.6 kg, T_2: 15.4 kg and T_3: 15.4 kg). After 105 days of the experiment the average body mass of the pigs in group T_1 was significantly ($P<0.001$) lower (82.3 kg) than that of animals in groups T_2 and T_3 (94.5 and 96.0 kg, respectively).

Along with clinical manifestations of parakeratosis the animals of group T_1 suffered from lower productivity (i.e. 16-17% lower body mass gain and 3-4% poorer feed conversion) than the animals of groups T_2 and T_3. There were no significant differences between groups T_2 and T_3 concerning feeding variables.

Ca, P and Zn in serum. No significant differences between the sexes of the pigs were detected for serum Ca, P and Zn concentrations and their ratios during the trial (data not shown).

Table 2 gives data on the concentration of calcium, phosphorus and zinc in blood serum and their ratios. No significant differences for calcium concentration were found between animals with no Zn supplement and those supplemented with either inorganic or organic zinc on days 0, 28 and 105, whereas on day 56 animals without a Zn supplement (group T_1) had a significantly higher mean serum concentration of Ca ($P<0.02$) compared to pigs supplemented with Zn-methionate (group T_3). Since the time profiles for Ca and Zn were completely different (Fig.1), the observed difference in Ca concentration may be assumed to be a weak transitory effect of supplementary zinc from an organic source. Swine of all groups (T_1, T_2 and T_3) showed significantly higher serum calcium concentration ($P<0.05$) at the beginning of the experiment and on day 56 than on days 28 and 105.

Similarly, the mean concentrations of serum phosphorus were not significantly different between groups T_1-T_3 on days 0, 28 and 56. At the end of the trial P returned to the starting values in groups T_2 and T_3, whereas P in group T_1 continued to rise. On day 105 pigs in group T_1 showed a significantly higher mean concentration of P ($P<0.02$) than the pigs group T_3. A significantly higher concentration of phosphorus ($P<0.05$) with in group T_1 was found only on day 56 *vs*. time 0. Animals of group T_2 showed significantly higher concentrations in the period 28-56 days ($P<0.05$) compared to the beginning and the end of the trial. In group T_3 the maximum concentration was observed on day 56, which was significantly higher ($P<0.005$) than any value at the other time-points.

In animals of all trial groups maximum zinc concentration appeared on day 28. At this point significant differences in zinc level occurred between all trial groups ($P<0.05$) and lasted for another month. In groups T_1 and T_3 the difference between day 28 and the other time points was significant throughout the trial. Unexpectedly, the significance level of these differences was more pronounced in group T_1 ($P<4\times10^{-8}$) than in group T_3 ($P<0.02$). This suggests a possible self-regulatory system being effective in group T_1. In group T_2 significant rise was evident between days 0 and 28 ($P<1\times10^{-6}$) with levelling off towards the end of experiment. The effect of the dietary zinc supplementation was evident on days 28, 56 and 105 when the pigs of the group T1 showed a significantly lower mean

concentration of zinc than the animals of the second and the third experimental groups ($P<0.02$). The concentration of serum zinc in group T_2 was significantly higher than that in group T_3 only on day 56 ($P<0.05$).

Table 2. Calcium, phosphorus and zinc in serum and their molar ratios

Day	Parameter (mmol/L)	Group of animals*, **		
		T_1	T_2	T_3
		X±SD	X±SD	X±SD
0	Ca	1.70±0.12 (8)^{ab}	1.65±0.16 (5)^{efg}	1.77±0.13 (11)^{jk}
	P	2.80±0.41 (7)^{a}	2.71±0.11 (5)^{bc}	2.83±0.22 (7)^{f}
	Zn	9.48±1.39 (13)^{abcA}	10.91±2.20 (14)^{ghiB}	12.55±2.34 (14)^{jklAB}
	P/Zn	317.70±67.5 (7)^{abcA}	240.90±58.3 (5)^{gh}	222.00±42.5 (7)^{jklA}
	P/Ca	1.63±0.22 (7)^{a}	1.66±0.19 (5)^{d}	1.60±0.09 (7)^{gh}
	Ca/Zn	194.80±26.5 (7)^{abAB}	147.60±45.0 (5)^{fghA}	139.40±27.0 (11)^{klmB}
28	Ca	0.85±0.52 (13)^{ac}	1.01±0.55 (14)^{eh}	1.15±0.58 (10)^{jl}
	P	3.03±0.27 (13)	2.95±0.21 (13)^{bd}	2.87±0.20 (12)^{g}
	Zn	17.15±1.92 (13)^{adeCD}	22.19±4.72 (13)^{gCE}	37.86±23.57(14)$^{\mathbf{jmnDE}}$
	P/Zn	180.80±26.7 (12)^{adeBC}	137.60±26.6 (13)^{giB}	101.70±62.6 (12)^{jmC}
	P/Ca	4.22±1.92 (12)^{abA}	3.93±1.99 (13)^{de}	2.71±1.24 (9)^{A}
	Ca/Zn	46.50±28.7 (12)^{acd}	48.70±34.1 (13)^{fi}	41.40±28.4 (10)^{kn}
56	Ca	1.89±0.18 (14)^{cdA}	1.76±0.14 (14)^{fhi}	1.67±0.14 (13)^{lmA}
	P	3.15±0.60 (14)^{a}	3.21±0.28 (14)^{cde}	3.39±0.21 (14)^{fgh}
	Zn	4.21±1.02 (14)^{bdfFG}	19.55±2.16 (14)^{hFH}	17.93±2.19(14)^{kmGH}
	P/Zn	779.40±190.6(14)^{bdfDE}	165.10±16.0 (14)^{hiDF}	191.90±26.6(14)^{kmnEF}
	P/Ca	1.68±0.33 (14)^{bcB}	1.83±0.22 (14)^{ef}	2.03±0.17 (13)^{giB}
	Ca/Zn	477.90±141.3(14)^{bceCD}	91.30±13.0 (14)^{gijC}	93.00±13.7 (13)^{lnoD}
105	Ca	1.06±0.53 (9)^{bd}	0.94±0.53 (11)^{gi}	1.03±0.49 (13)^{km}
	P	3.63±0.91 (10)^{A}	2.88±0.30 (11)^{e}	2.73±0.22 (13)^{hA}
	Zn	6.01±2.19 (14)^{ceflJ}	20.43±4.06 (14)^{il}	18.33±4.55 (13)^{lnJ}
	P/Zn	648.90±238.2(10)^{cefGH}	149.30±32.0 (11)^{G}	148.30±36.4 (12)^{lnH}
	P/Ca	4.68±3.18 (9)^{c}	3.56±1.83 (10)^{f}	2.91±1.16 (12)^{hi}
	Ca/Zn	177.70±92.2 (9)^{deEF}	48.50±29.7 (11)^{hjE}	58.20±25.2 (12)^{moF}

* Each capital letter refers to a significant difference ($P<0.05$) between trial groups and each small letter refers to a significant difference ($P<0.05$) between trial time-points, for each element or molar ratio.
** Number of independent analyses in parentheses.

The mean time profiles of Ca, P and Zn in sera of all groups during the experiment are also presented in Figs. 1a-c.

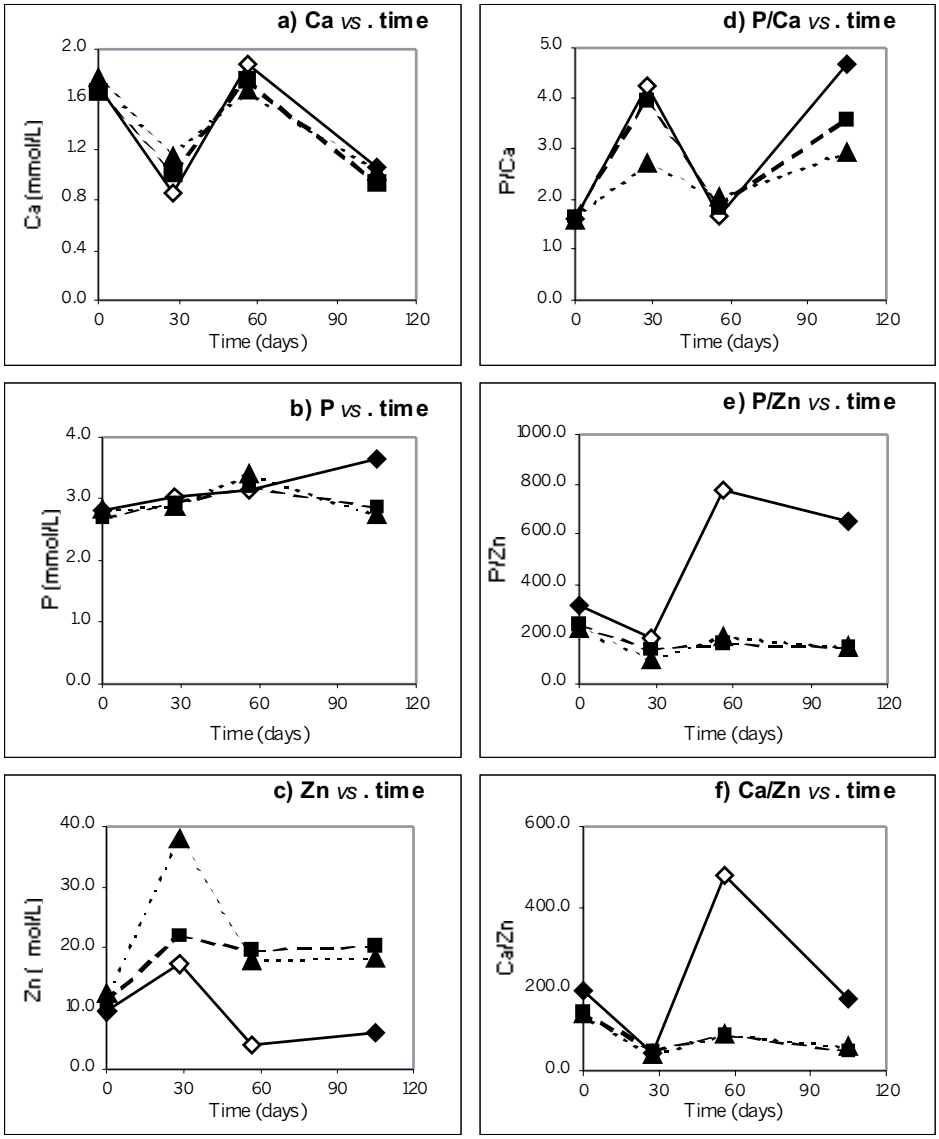

Figure 1. Comparative time profiles for Ca, P and Zn in swine sera and their molar ratios: group T_1: no parakeratosis (–♦–), 100% parakeratosis (–✧–);group T_2 (--■--);group T_3 (···▲···).

Ca, P and Zn molar ratios.

P/Ca values followed a zigzag time profile inverse to that of serum Ca in all groups (Table 2). However, a significant decrease of P/Ca values was evident on day 28 ($P<0.05$) and a significant increase on day 56 ($P<0.005$) in T_3 *vs*. T_1. No significant changes of P/Ca ratio were observed when inorganic zinc was supplied. Along the time axis a significant increase of P/Ca ratio in group T_3 was detected only between time zero and day 56 ($P<0.001$) and 105 ($P<0.02$).

Significant differences for P/Zn values were observed between groups T_1 and T_3 ($P<0.01$) and between groups T_1 and T_2 ($P<0.002$) throughout the experimental period. The experimental groups were significantly different one from the other only at day 56. However, both Zn-supplemented groups followed almost identical time profiles. As expected P/Zn values were dominantly governed by serum Zn concentration. This was evident through variability of P/Zn values even at time 0 as well as through the characteristic time profile of P/Zn in group T_1 which was inverse to that of zinc.

Again, irrespectively of the type of supplemented zinc significantly lower Ca/Zn ratios occurred on days 56 and 105 in groups T_3 and T_2 vs. group T_1 respectively $P<0.02$ and $P<2x10^{-3}$. Similar effects of each type of dietary zinc on the Ca/Zn ratio were seen from the almost identical ratio vs. time plots. For example, significant differences between day 0 and day 105 were observed in both groups T_2 and T_3 ($P<0.02$). Ca/Zn ratios were found to be directed both by Ca and zinc levels in serum. However, the variability of Ca/Zn at $t=0$ and during the experiment suggests a dominant influence of zinc concentration. Nevertheless the addition of inorganic zinc caused a marked and expected inversely linear correlation between Ca/Zn ratio and P. Namely, the correlation coefficient changed from -0.16 on day 28, via -0.50 on day 56 to -0.72 on day 105.

The mean time profiles of P/Ca, P/Zn and Ca/Zn molar ratios in sera of all groups during the experiment are presented in Figs. 1d-f.

Clinical evidence. After the 30-day pre-trial feeding with a low zink diet namely on the first day of the trial no symptoms of parakeratosis were noticed in any experimental animal. After 22 days on the starter diet all animals from group T_1 showed clinical evidence of typical parakeratosis (lower food consumption, itching and red swellings 1-2 cm wide, with crusts on the skin, particularly on the belly, with a tendency to become confluent). After 56 days of the experiment T_1 animals still suffered from strong itching and the skin of the whole body was covered with keratinous crusts. Pigs in groups T_2 and T_3 fed with inorganic and organic zinc, respectively, showed no signs of parakeratosis during the same period.

In the period from day 80 to day 90 itching and parakeratotic changes on the skin of T_1 animals disappeared. This is the period when serum Ca dropped sharply and P continued to increase in T_1. In the same period and in the same group Ca/Zn and P/Zn ratios dropped markedly whereas P/Ca values sharply increased.

DISCUSSION

At the beginning of the trial (day 0) no statistically significant differences in concentration of Ca in serum were found between the groups. The same was true for the period after feeding the starters (day 28) which contained the same concentrations of Ca (0.78%) but different concentrations of zinc (T_1: 45.50 mg/kg, T_2: 128.00 mg/kg, T_3: 101.80 mg/kg). However, it should be pointed out that in this period (day 23) clinical manifestations of parakeratosis appeared in T_1 group animals in the form of redness, damage and blisters on the skin of the whole body. After feeding with the growers (day 56), which also contained the same concentrations of Ca (0.72%) but different concentrations of zinc (T_1: 38.00 mg/kg, T_2: 124.26 mg/kg, T_3: 80.30 mg/kg), animals of group T_1 showed a significantly higher mean concentration of serum Ca than T_3 swine, which were supplemented with zinc from anorganic source. In this period all T_1 animals still had keratinous crusts on the whole skin. After feeding with the finishers (day 105) which contained the same concentrations of calcium (0.60%) but different concentrations of zinc (T_1: 31.00 mg/kg, T_2: 115.20 mg/kg, T_3: 62.33 mg/kg), no significant differences for serum Ca were observed between the groups. In the same period, namely between days 80 and 90 keratinous crusts in T_1 pigs gradually disappeared. Therefore no clinical evidence of hyperkeratosis was found at the end of the experiment (day 105).

In this investigation the serum Ca ranged between 0.85 and 1.89 mmol/L. According to the physiological values for Ca in pigs of 1.8-4.0 mmol/L as reported by Swenson (1975), Imlah and McTaggart (1977), Scheunert and Trautmann (1987), Odink *et al.* (1990), Gomerčić and Gomerčić (1996), Kaneko *et al.* (1997) and Underwood and Suttle (1999) our values of Ca were at the lower limit or even in the hypocalcemic region during the trial. Possible reasons might be reduced food intake and the composition of the diet, which was relatively low in Ca but high in P.

Since the same upward trend of P values was observed in all animals regardless of dietary zinc supplementation, there were no statistically significant differences between the trial groups at the beginning of the experiment (day 0), or after feeding with starters (day 28) and growers (day 56) which contained the same concentrations of P (0.65 and 0.53%, respectively) but different concentrations of zinc (see Table 1). After feeding with finishers (0.38% P) P in the serum of group T_1 continued to rise but returned to initial values in T_2 and T_3. Namelu,significant differences appeared during feeding with finishers.

Mean concentrations of P in the sera of all groups ranged between 2.71 and 3.63 mmol/L which is in the physiological range of 1.1-3.1 mmol/L reported by Odink *et al.* (1990), Gomerčić and Gomerčić (1996), Kaneko *et al.* (1997) and Underwood and Suttle (1999) but slightly higher than the values reported by Imlah and McTaggart (1977) and Scheunert and Trautmann, 1987) (1.3-2.5 mmol/L).

In order to detect the possible nutritional effects of various amounts and sources of zinc it was necessary to empty Zn reservoirs in the body (liver, bones and other tissues) in all animals (groups T_1-T_3). This was accomplished by the pre-trial feeding regime lasting for 30 days when the animals were fed starter (45.50

mg Zn/kg) with no extra Zn supplement. At the beginning of the trial (t=0) the mean concentration of Zn in serum ranged between 9.48 and 12.55 μmol/L, which was higher than the values reported for zinc deficiency (1.8-7.6 μmol/L) according to Hoekstra *et al.* (1956, 1967) but in the lower physiological level according to Puls (1990), Hoekstra *et al.* (1967), Whitenack *et al.* (1978), Miller *et al.* (1979) and Wedekind *et al.* (1994) (8.4-22.9 μmol/L). The low concentration of zinc at the beginning of experiment was not accompanied by reduced food consumption and other clinical indicators of zinc deficiency.

The pigs fed starter only, namely at t=0, showed unexpected variability of serum zinc values (see Table 2). Moreover, the animals demonstrated a kind of a self-regulatory mechanism throughout the trial. Thus, pigs which were not supplemented with extra dietary zinc (T_1) followed the same time contour as groups T_2 and T_3 reaching a maximum after 28 days and levelling off up to the end of experiment. This was probably due to the pronounced ability of animals to recruit zinc from all the reservoirs in the body in order to maintain as high a level of serum zinc as possible. Such behaviour has already been noticed by Hill *et al.* (1986), Wedekind *et al.* (1994) and confirmed by Rupić *et al.* (1997).

Starting with day 28 up to the end of the trial (day 105) significantly higher mean serum zinc concentrations occurred in both groups T_2 and T_3 than in group T_1. Moreover, by the end of the 1st month of the trial the pigs which received half as much zinc but in the form of Zn-methionate (group T_3) showed a significantly higher mean serum concentration of Zn than those receiving $ZnSO_4$ (group T_2) (see Tables 1 and 2). These data indicate that the organic source of Zn was much better exploited than the inorganic source in the period of fast growth and body mass gain (up to day 56). After 22 days of treatment without zinc supplement (basal group T_1) typical parakeratosis occurred but the pathological changes on the skin disappeared just before the end of the experiment. No symptoms of parakeratosis appeared in either of the Zn-supplemented groups. During the period of slower growth Zn from Zn-methionate in half the amount given as $ZnSO_4$ was sufficient to meet the metabolic requirements of the body, preserve good health and support very good productivity in fattening pigs. The basal group (T_1) was fed a marginal level of Zn (see Table 1) because the diet(s) were based on vegetable ingredients in which a significant amount of Zn was bound by phytate (NRC, 1998). According to Shatzman and Henkin (1981) Zn-deficient diets lead to reduced appetite because Zn is a component of gustin, a protein involved in taste activity. Hoefer *et al.* (1960) investigated the influence of different ratios of calcium, zinc, iron and copper in the diet on the respective concentrations in blood serum of swine. They observed that feeding with a meal containing only 28.2 mg Zn/kg caused parakeratosis in almost 60% of swine in the group. Forenbacher (1964) stated that parakeratosis is a symptom of metabolic disbalance in fattening pigs caused by the deficiency of zinc. However, along with the deficiency of zinc this disorder may be induced by dry fodder mixes and particularly by an excess of Ca in the feed leading to the biological antagonism between Ca and Zn. The same author stressed that experimental fattening with diets containing 1.4% Ca led to parakeratosis in 100% of cases. Pond and Jones (1964) and Newland *et al.* (1958) claimed that the intestinal absorption of zinc in swine was decreased in the

presence of high levels of Ca and phytates in the meal, namely when the major sources of proteins are soy beans and maize. An excess of vitamin D has the same effect. Greer *et al.* (1980) mentioned that the ratios between iron, copper, cadmium and zinc influence the appearance of parakeratosis. Iron and copper behave as antagonists of zinc and their effect depends on the amount in the diet. Excessive zinc leads to excretion of copper and a deficiency of iron, whereas addition of 250 μg Cu/kg of meal increased the absorption of zinc by about 35%. The mentioned concentration of copper led to symptoms of Cu toxicity if no zinc was added. Doyle (1980) believed that disorders of Zn metabolism may be genetically determined (by a recessive gene). It has been also demonstrated that excessive Fe (Fe:Zn, 5-10:1) inhibits absorption of Zn in humans regardless of its form (Valberg *et al.*, 1984). In our experiments (Table 1) the ratio Fe:Zn was 5.4 in the basal diet. Along with the aforementioned factors the supplementation of pigs with minerals depends on the age, sex, physiological state, infections with various microorganisms, stress, temperature of the environment, and other conditions.

During the last experimental period the serum zinc level settled in all groups, at the upper physiological levels in T_2 and T_3 groups and at hypozincaemic level in group T_1. The characteristic time profile was present in all three experimental groups but at different serum Zn levels. The maximum zinc level occurred in all groups after the 1st month of experimental fattening. The Zn level decrease recorded in groups T_2 and T_3 afterwards could be due to increased elimination of Zn from the body. As far as group T_3 is concerned diminishing Zn values may be also due to the lower concentration of Zn from Zn-methionate in the consecutive diet.

A significant drop of P/Ca from 4.2 in T_1 to 2.7 in T_3 had taken place on day 28 when the maximum concentration of Zn in serum (37.86 μmol/L) was achieved.

Both Ca/Zn and P/Zn ratios showed similar time contours: a persistent and significant decrease of both ratios was observed as a consequence of Zn supplementation irrespectively of the zinc source. Moreover, in both cases group T_1 differed significantly from group T_2 and T_3, while groups T_2 and T_3 followed practically the same profiles. This suggests that both ratios were primarily governed by the serum zinc levels: the higher the zinc level the lower the P/Zn or Ca/Zn ratio. As expected, both P/Zn and Ca/Zn were strongly increased by feeding the non-supplemented diet. Since the diets applied differed in concentration of zinc only the effects observed could be primarily accounted for by the extra supplementation of zinc. Therefore these ratios may be used as reliable indicators of Zn status in swine: P/Zn $\leq$300 and Ca/Zn $\leq$200 would indicate the physiological concentrations of zinc in the serum of pigs.

No correlation between the appearance of parakeratotic changes on the skin and the concentration of Ca or P in serum could be found. Appearance of parakeratosis should be expected at the low levels of zinc and relatively high Ca/Zn and P/Zn ratios. The fact that animals supplemented with extra dietary zinc did not suffer parakeratotic changes and showed significantly higher productivity in terms of significantly higher final body mass, body mass gain, food consumption and feed conversion compared to the animals with no supplementation, supports conclusions on the protective role of zinc (Pond and

Jones, 1964; Forenbacher, 1964; Hoekstra *et al.*, 1967; Miller *et al.*, 1979; Mills, 1987; Mertz, 1987; Underwood, 1981; Rupić *et al.*, 1997, and others). However, the effects observed during the study were occasionally controversial. For example, the symptoms of parakeratosis disappeared in group T_1 regardless of the fact that it was not supplemented with extra dietary zinc and that Zn level was pathologically low (6.01 μmol/L). This phenomenon could be interpreted from several aspects. Some kind of slow self-regulatory mechanism may have taken part in the unexpected disappearance of symptoms of parakeratosis between 80 and 90 days of the trial. The disappearance of pathological parakeratotic changes on the skin of the T_1 pigs during last two weeks of the experiment could be also due to the slower processes of growth and formation of body mass (proteins) because T_1 animals primarily converted the food the fat, as well as due to increased food consumption which met the basic metabolic requirements for Zn. The disappearance of parakeratotic symptoms in T_1 animals coincided with the reduction of serum Ca and increase of serum P, as well as in the reduction of Ca/Zn and P/Zn and the increase of P/Ca.

Accordingly, all the data mentioned above appear to lead to the conclusion that a diet with properly balanced minerals is vital for maintaining high productivity and good health of swine. However, some of the controversial effects observed open new questions or reopen known ones on regulatory mechanisms operating in nutritionally exhausted pigs.

Address for correspondence:
Prof. Dr. Vlatko Rupić
Faculty of Agriculture
University of Zagreb
Svetošimunska cesta 25
HR-10000 Zagreb, Croatia
e-mail: vlatko.rupic@zg.htnet.hr

REFERENCES

1. *AOAC*, 1984, Official Methods of Analysis, 14th ed, Arlington: Association of Official Analytical Chemists.
2. *Butrimovitz GP, Purdy WC*, 1977, The determination of zinc in blood plasma by atomic absorption spectrometry, *Anal Chim Acta,* 94, 63-73.
3. *Connerty HV, Briggs AR*, 1966, Determination of serum calcium by means of orthocresolphthalein complexone, *Am J Clin Pathol*, 45, 290-6.
4. *Doyle JJ*, 1980, Genetic and nongenetic factors affecting the elemental composition of humans and other animal tissue, *J Anim Sci*, 50, 1173-83.
5. *Endres DB, Rude RK*, 2001, Mineral and bone metabolism, In: Burtis CA, Ashwood ER, editors, Tietz Fundamentals of Clinical Chemistry, 4th ed, Philadelphia: WB Saunders Company, 795-821.
6. *Fiske CH, Subbarow Y*, 1925, The colorimetric determination of phosphorus, *J Biol Chem*, 66, 375-400.
7. *Forenbacher S*, 1964, *Unutrašnje bolesti domaćih životinja* (III dio), Zagreb: Sveučilište u Zagrebu, 122-3 Š*Internal Diseases of Domestic Animals*Ć.
8. *Gomerčić H, Gomerčić V*, 1996, Neki biološki pokazatelji različitih vrsta životinja, U: Veterinarski priručnik, 5th ed, Zagreb: Medicinska naklada, 1269 ŠSome biological indicators of different kinds of animals, In: *Veterinary Handbook*Ć.

9. *Greer EB, Lewis CE, Crof MG*, 1980, Mineral and vitamin supplementation of diets for growing pigs, *Nutr Abstr Rev*, 50, 147.
10. *Hill DA, Poe ER, Lewis AJ, Crenshaw JD*, 1986, Zinc-amino acid complexes for swine, *J Anim Sci*, 63, 121-30.
11. *Hoefer JA, Miller ER, Ullrey DE, Ritche HD, Luecke RW*, 1960, Interrelationships beetween calcium, zinc, iron and copper in swine feeding, *J Anim Sci*, 19, 249-59.
12. *Hoekstra WG, Lewis PK, Jr, Phillips PH, Grummer RH*, 1956, The relationship of parakeratosis, supplemental calcium and zinc to the zinc content of certain body components of swine, *J Anim Sci*, 15, 752-64.
13. *Hoekstra WG, Faltin EC, Lin CW, Roberts HF, Grummer HR*, 1967, Zinc deficiency in reproducting gilts fed a diet high in calcium and its effect on tissue zinc and blood serum alkaline phosphatase, *J Anim Sci*, 26, 1348-57.
14. *Imlah P, McTaggart HS*, 1977, Haematology of the pig, In: Archer RK, Jeffcott LB, editors, Comparative Clinical Haematology, Oxford: Blackwell Scientific Publications, 272-85.
15. *Kaneko JJ, Harvey JW, Bruss ML*, 1997, *Clinical Biochemistry of Domestic Animals*, 5th ed, San Diego: Academic Press, 890-3.
16. *Kirchgessner M, Hartel J*, 1977, Zur intermediären Zinkverfügbarkeit 15 verschiedener Zinkverbindungen. *Ztsch Tierphysiol Tierenährung Futtermitkd*, 38, 138-46.
17. *Luecke RW, Hoeffer AJ, Brammellm SW, Smidt AD*, 1957, Calcium and zinc in parakeratosis of swine, *J Anim Sci*, 16, 3-11.
18. *Mertz W*, 1987, *Trace Elements in Human and Animal Nutrition*, 5th ed, San Diego: Academic Press, 1-480.
19. *Miller ER, Stowe HD, Ku PK, Hill GM*, 1979, Copper and zinc in swine nutrition, In: National Feed Ingredients Association Literature: Review on Copper and Zinc in Animal Nutrition, West Des Moines: Natl Feed Ingredient Assoc, 1-139.
20. *Milne DB*, 1999, Trace elements, In: Burtis CA, Ashwood, ER, editors, Tietz Textbook of Clinical Chemistry, 3rd ed, London: WB Saunders Company, 1037-41.
21. *Mills CF*, 1987, Biochemical and physiological indicators of mineral status in animals: Copper, cobalt and zinc, *J Anim Sci*, 65, 1702-11.
22. *National Research Council*, 1998, Nutrient Requirements of Swine, 10th ed, Washington, DC: National Academy of Science, 189.
23. *Newland HW, Ullrey DE, Hoefer JA, Luecke RW*, 1958, The relationship of dietary calcium to zinc metabolism in pigs, *J Anim Sci*, 17, 886-92.
24. *Odink J, Smeets JFM, Visser IJR, Sandman H, Snijders JMA*, 1990, Hematological and clinicochemical profiles of healthy swine and swine with inflammatory processes, *J Anim Sci*, 68, 163-70.
25. *Pond WG, Jones JR*, 1964, Effect of level of zinc in high calcium diets on pigs from weaning through one reproductive cycle and on subsequent growth of their offspring, *J Anim Sci*, 23, 1057-60.
26. *Pond WG, Church DC, Pond KH*, 1995, Zinc, In: Basic Animal Nutrition and Feeding, 4th ed, New York: John Wiley and Sons, 190-4.
27. *Puls R*, 1990, Mineral Levels in Animal Health: Diagnostic Data, Vancouver: Clearbook, Sherpa International.
28. *Rupić V, Ivandija L, Luterotti S, Dominis-Kramarić M*, 1997, Influence of inorganic and organic dietary zinc on zinc concentration in blood serum, bones and hair and on catalytical activity of some serum enzymes in pigs, *Acta Vet Brno,* 66, 7-17.
29. *Sanstead HH*, 1994, Understanding zinc: recent observations and interpretations, *J Lab Clin Med*, 124, 322-7.
30. *Scheunert A, Trautmann A*, 1987, Lehrbuch der Veterinär-Physiologie, Berlin und Hamburg: Verlag Paul Parey, 93-119.
31. *Shatzman AR, Henkin RI*, 1981, Gustin concentration changes relative to salivary zinc and taste in humans, *Proc Natl Acad Sci USA*, 78, 3867-75.
32. *Smith JC, Jr, Butrimovitz GP, Purdy WC*, 1979, Direct measurement of zinc in plasma by atomic absorption spectroscopy, *Clin Chem*, 25, 1487-91.

33. *Swenson JM*, 1975, Djuksova fiziologija domaćih životinja, 8th ed, Sarajevo: Svjetlost ŠDjuks's Physiology of Domestic AnimalsĆ.
34. *Tucker HF, Salmon WD*, 1955, Paraceratosis or zinc deficiency disease in the pig, *Proc Soc Exp Biol Med*, 88, 613-6.
35. *Underwood EJ*, 1981, Zinc, In: The Mineral Nutrition of Livestock, London: Commonwealth Agricultural Bureau, 135-46.
36. *Underwood EJ, Suttle NF*, 1999, The Mineral Nutrition of Livestock, 3rd ed, New York: CABI Publishing, 67-149, 477-513.
37. *Valberg LS, Flangan PR, Chamberlain MJ*, 1984, Effect of iron, tin, and copper on zinc absorption in humans, *Am J Clin Nutr*, 40, 536-44.
38. *Wedekind KJ, Lewis AJ, Giesemann MA, Miller PS*, 1994, Bioavailability of zinc from inorganic and organic sources for pigs fed corn-soybean meal diets, *J Anim Sci*, 72, 2681-9.
39. *Whitenack DL, Whitehair CK, Miller ER*, 1978, Influence of enteric infection on zinc utilization and clinical signs and lesions of zinc deficiency in young swine, *Am J Vet Res*, 39, 1447-57.

KALCIJUM, FOSFOR, CINK I NJIHOVI ODNOSI U SERUMU TOVNIH SVINJA HRANJENIH UZ RAZLIČITE DODATKE CINKA

RUPIĆ V, LUTEROTTI SVJETLANA, ČEPELAK IVANA, REKIĆ BRANKICA, GRBEŠA D i KNEŽEVIĆ M

SADRŽAJ

Ispitivan je uticaj cinka dodatog u hranu koja sadrži stalne koncentracije kalcija i fosfora, na serumske koncentracije kalcija, fosfora i cinka i njihove molarne odnose u tovnih meleza German Landrace x Piétren x Large White x Swedish Landrace. Nađena je značajno bolja bioraspoloživost cinka iz Zn-metionata nego iz $ZnSO_4$ u periodu naglog rasta i prirasta telesne mase. Karakteristični vremenski profil za Zn vidljiv je u svim oglednim grupama ali pri različitim koncentracijama cinka.

Nije uočen naglašen uticaj organskog ili anorganskog cinka dodanog u hranu na serumske koncentracije Ca i P. Štaviše, nije nađen ni ustaljeni trend P/Ca vrijednosti. Cink dodan u obliku kelata ili anorganske soli doveo je do trajnog pada vrednosti odnosa P/Zn i Ca/Zn. S obzirom na to ovi odnosi mogu se koristiti kao pouzdani pokazatelji statusa cinka u svinja: P/Zn ≤300 i Ca/Zn ≤200 ukazuju na fiziološke koncentracije cinka u serumu svinja. Nasuprot tome, pojava parakeratoze može se očekivati kod niskih koncentracija cinka i relativno visokih odnosa Ca/Zn i P/Zn.