INFLUENCE OF INORGANIC AND ORGANIC DIETARY ZINC ON ITS CONCENTRATION IN BLOOD SERUM, BONES AND HAIR AND ON CATALYTICAL ACTIVITY OF SOME SERUM ENZYMES IN PIGS

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Abstract

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The influence of dietary zinc originating from either inorganic or organic source on zinc-depleted pigs was investigated. Within the 30 d period preceding the trial, all the animals (German Landrace × Piétrain × Large White × Swedish Landrace weaned crossbreds) were depleted of zinc and divided into three groups, 14 animals in each. During the experimental fattening period (105 d), the control group received no extra zinc, whereas the two trial groups were fed a diet with zinc supplement either as inorganic salt ($ZnSO_4$) or metalo-organic chelate (zinc methionate). On average, the respective supplements contained 84.3 mg $Zn.kg^{-1}$ diet and 40.9 mg $Zn.kg^{-1}$ diet.

Evaluation of the nutritional effects of zinc was based on its concentration in the blood serum, bone and hair. Moreover, the effect of zinc on catalytical activity of alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase and gamma-glutamyltransferase in serum was studied. A decrease of catalytical activity of both aspartate aminotransferase and alanine aminotransferase was found along with signs of parakeratosis on the skin of control animals but no correlation with serum zinc was established. In contrast to this finding, a linear correlation between catalytical activity of alkaline phosphatase and of gamma-glutamyltransferase and serum zinc level was found with the respective correlation coefficients of 0.90 and -0.72.

Nutritional efficiency of diet zinc was evidenced by both higher ($P < 0.05$) zinc level in blood serum, bone and hair and by the state of hepatobiliary tract and bones, and favourable performance results of trial animals compared to controls. None of trial animals suffered from any health disturbances. Progressive parakeratosis appeared, however, in control animals and lasted during the second month of the trial. It was accompanied by deterioration of performance data of the affected animals.

Our data suggest that bioavailability of zinc from the chelate was better than of that derived from inorganic salt, particularly in the period of rapid growth. This presumbaly resulted in reduced elimination of zinc. Based on better nutritional efficiency in pigs and on ecological benefits, zinc methionate should be preferred to $ZnSO_4$ as a zinc containing diet additive.

Growth, pigs, zinc sulphate, zinc methionate, bioavailability, nutrition

The improvement of body mass gain and carcass quality are among major goals of all pork producers. One of the ways to achieve this is to provide the animals with necessary minerals from the sources of favourable bioavailability. Circulation of zinc and its biological importance in animals (cellular replication, lung tissue CO_2 release, sexual maturation, wound healing, fertility and reproduction) have been extensively investigated. Zinc is an essential trace element, and it is an integral part of some 300 enzymes (carbonic anhydrase, alkaline phosphatase, alcohol dehydrogenase, RNA and DNA polymerases, 5-nucleotidase, etc.) which take part in protein synthesis, metabolism of carbohydrates and nucleic acids (Milne 1994). It is assumed that Zn derived from compounds with amino acids stimulates

production in animals. Accordingly, Wedekind et al. (1992) pointed to better bioavailability of zinc methionate (ZnMET) compared to that of its inorganic compounds in chickens. Moreover, Herrick (1989) demonstrated an improved body mass gain in fattening cattle by the use of ZnMET in diet. Hartfield et al. (1995) brought evidence of increased body mass gain of 6-9% in weaned lambs supplemented with ZnMET. Rust (1995) and Berrie et al. (1994) concluded on improved carcass quality in fattening bull-calves and lambs fed diets with ZnMET. We are particularly interested in the influence of different sources of dietary zinc on performance data and state of health of pigs. There is still no consensus among the investigators considering the bioavailability of inorganic and organic sources of zinc in pigs. Hill et al. (1986) reported on better production results in piglets fed diet with organic zinc, whereas Wedekind et al. (1994) failed to notice such differences.

The aim of our study was to evaluate the bioavailability of diet zinc originating from different chemical sources (inorganic salt and metalo-organic chelate) based on the concentration of zinc in blood serum, bone and hair of growing pigs. As an indicator of the state of hepatobiliary tract and bones, the catalytical activity of alkaline phosphatase (EC 3.1.3.1; AP), aspartate aminotransferase (EC 2.6.1.1; AST), alanine aminotransferase (EC 2.6.1.2; ALT) and gamma-glutamyltransferase (EC 2.3.2.2; GGT) in serum was monitored as well.

Materials and Methods

Animals and diets

A total of 42 German Landrace x Piétrain x Large White x Swedish Landrace weaned crossbreds was used in the experiment. Pigs were kept in boxes, seven pigs per box (a total of six boxes). There were seven males and seven females in each group. After weighing, the piglets were divided into three groups: control group, C (n = 14), trial group T_1 (n = 14), and trial group T_2 (n = 14). The average starting body mass for all groups of animals was 15.5 kg (see Table 2). All groups in trial contained pigs from seven different litters born on the same day. Equal number of pigs from one litter (one male and one female) were included in each group. The animals were identified by numbers 1 to 42, tattooed on the right ear. The male piglets were castrated at the age of 14 d.

All groups of animals were kept in the same microclimatic conditions. During the experimental period the average air temperature was 18.0 °C and relative humidity 78.8%. There was a forced air circulation in the animal room. The state of health in pigs was checked daily.

Pigs were offered the diets and water *ad libitum* by means of automatic feeding and watering systems. Composition of fodder mixes is given in Table 1.

Within the 30 d period preceding the experiment, the piglets became accustomed to the environment and were fed a starter diet (193.10 $g.kg^{-1}$ of proteins) with no Zn supplement. By chemical analysis of the starter, 45.5 mg $Zn.kg^{-1}$ was determined. This feeding regime was intended to exhaust the Zn depots in piglets. During the subsequent experimental fattening (105 d), all groups were fed the same basic fodder mix (Table 1) in the following sequence: starter (up to 32 kg body mass), grower (up to 50 kg body mass), and finisher (up to 90 kg body mass).

Control animals were fed the basic fodder mix containing on average 36.7 mg $Zn.kg^{-1}$ diet throughout the trial. The T_1 group was given an extra Zn in the form of $ZnSO_4$: the average addition of Zn from $ZnSO_4$ was 84.3 mg $Zn.kg^{-1}$- fodder (starter: 82.5, grower: 86.3, finisher: 84.2 mg $Zn.kg^{-1}$ diet). The T_2 trial group was given additional Zn in ZnMET: an average addition of Zn from ZnMET was 40.9 mg $Zn.kg^{-1}$ fodder (starter: 56.3, grower: 42.3, finisher: 31.3 $mg.kg^{-1}$ diet). $ZnSO_4$ and ZnMET were mixed into vitamin-mineral premixes which were added to the respective starter mix in a concentration of 1% and to grower and finisher mixes at a concentration of 0.5%.

We measured the following feeding variables: starting and finishing body mass, body mass gain, food intake and feed conversion. They were determined after 4, 8, 12 and 15 weeks of experiment. To assess the nutritional effects of zinc supplemented to fodder mixes, blood serum biochemical parameters in experimental and control pigs were compared. Food consumption was measured for a whole group of pigs and the body mass for each animal separately.

As sources of zinc, $ZnSO_4xH_2O$ (B.B.V. Chemie, Duisburg, Germany) and Zn-methionate (Pliva, Zagreb, Croatia) were used.

Sampling and analyses

All reagents used were of analytical grade. Deionized and then distilled water was used throughout.

During collection and handling of specimens disposable plasticware was used whenever possible. All glassware

Table 1
Ingredients and chemical composition of diets

Ingredients (% as fed)	Starter	Grower	Finisher
Corn	46.05	42.50	39.50
Barley meal	22.00	33.45	41.00
Wheat middlings	–	–	3.50
Soybean meal	19.00	13.00	9.50
Sunflower meal	-	6.00	-
Alfalfa meal	1.50	2.50	3.40
Fodder Yeast (vet.)	6.67	-	1.00
Fat	1.20	-	-
Iodized salt	0.30	0.35	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 ($g.kg^{-1}$)	885.60	892.00	888.00
Crude protein ($g.kg^{-1}$)	193.10	156.80	133.00
Crude fat ($g.kg^{-1}$)	37.50	30.00	26.00
Crude fibre ($g.kg^{-1}$)	37.70	48.10	40.20
Ca ($g.kg^{-1}$)	7.80	7.2	6.00
P ($g.kg^{-1}$)	6.50	5.30	3.80
Mn ($mg.kg^{-1}$)	46.00	48.70	52.37
Fe ($mg.kg^{-1}$)	315.00	178.26	139.40
Cu ($mg.kg^{-1}$)	32.50	20.24	12.30
Zn ($mg.kg^{-1}$) C	45.50	38.00	31.00
Zn ($mg.kg^{-1}$)† T_1	128.00	124.26	115.20
Zn ($mg.kg^{-1}$)# T_2	101.80	80.30	62.33

* Vitamin-mineral premixes contained:
Zn- free **VAM - SP** provided the following per kilogram: vitamin A, 1,000.000 IU; vitamin D_3, 100,000 ICU; 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; J, 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 **VAM - ST** provided the following per kilogram; vitamin A, 1,000.000 IU; vitamin D3, 100,000 ICU; vitamin E, 2,400 mg; vitamin K_3, 400 mg; vitamin B1, 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; J, 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 (A.O.A.C., 1984).

† After addition of $ZnSO_4$ to a vitamin-mineral premix.

\# Afer addition of ZnMET to vitamin-mineral premix.

was acid-washed (soaked in 1:1 HNO_3 for 24 hr 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. These tubes were used also for preparing sample dilution's.

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

Analysis of Zn concentration in blood serum was performed by flame atomic- absorption spectrometry (Perkin-Elmer 305B atomic absorption spectrophotometer, Perkin-Elmer, Norfolk, CAT, USA) under standard operating conditions. Samples were prepared by diluting sera with water in a volume ratio 1:4. The diluent used for the preparation of a blank and of standard solutions was 5 % (V/V) glycerol (Perkin-Elmer 1976; Butrimovitz and Purdy 1977; Smith et al. 1979). The accuracy of the method was checked upon lyophilised control serum Validate-A (Lot No. 101553, Organon Teknika, Eppelheim, Germany).

Determination of Zn in both bone and hair was done by graphite furnace atomic-absorption spectrometry (Unicam 920 AA spectrometer with Unicam GF 90 furnace, Unicam, Cambridge, UK). The samples were collected and treated as follows: At the end of the experiment, pigs from all groups were killed and the coccygeal vertebrae were collected for Zn analysis. The feet and tails were autoclaved at 120 °C for 20 min to facilitate the removal of muscle, skin and connective tissue. The bones were then dried at 105 °C overnight. The dried bones were then extracted with anhydrous ethyl ether for 48 hr. The dried, fat-free bones were than ashed at 720 °C for 3 hr. Bone samples were dissolved in 6 $mol.l^{-1}$ HCl and diluted appropriately for the Zn analysis. At the beginning of the experiment an average sample of hair was taken from all groups, and at the end of the experiment, the animals in each group were sampled. The same ashing procedure followed by dissolution in HCl as stated for bones was applied to hair samples as well.

Catalytical activities of AP, AST, ALT and GGT in blood serum were determined using Chronolab test reagents at 30 °C. Spectrometric measurements were performed on an automatic analyser (VP - ABBOT).

Animals employed in this study were maintained in facilities approved by the Croatian Association for Accreditation of Laboratory Animals Care and in accordance with current regulations and standards of the Croatian Ministry of Agriculture.

Statistics

All statistical analyses were performed using the GLM procedure of SAS (1989).

Results

Feeding variables

Mean values of feeding variables investigated over 105 d of experimental fattening are given in Table 2. No significant difference was detected in the body mass among the three groups at the beginning of the trial. During the trial pigs in groups T_1 and T_2 attained greater

Table 2
Average feeding variables after 105 d of the trial

Feeding variable	Group of animals*		
	C	T_1	T_2
Body mass ($x \pm \sigma$, kg)			
Starting	15.65 ± 1.82	15.41 ± 2.77	15.42 ± 2.31
Finishing	82.25 ±12.82	94.50 ± 8.87	95.97 ± 8.83
Index (%)	100	114.9	116.7
Daily mass gain ($x \pm \sigma$, g)	634.43 ± 120.15**	753.24 ± 97.57**	767.14 ± 83.90**
Index (%)	100	118.8	120.9
Daily food intake (x, kg)	2.08	2.38	2.45
Index (%)	100	114.4	117.8
Feed conversion (x, kg . kg^{-1})	3.28	3.16	3.19
Index (%)	100	96.3	97.3

* Number of pigs in each group, n = 14.
** Significant difference ($P < 0.05$) of average feeding variables between trial groups and the control group.

($P < 0.05$) body mass (by 14.9-16.7%) and body mass gain (by 18.8-20.9%) than the controls. Comparison of C group with T_1 and T_2 trial pointed also to the higher daily food intake (14.4-17.8 %) of the latter. Better feed conversion (by 2.7-3.7%) was also recorded in T_1 and T_2 than in C group. Fair but not significant improvements of feeding variables were evidenced in group T_2 if compared with group T_1.

Table 3 gives the data on Zn concentration in blood serum, bone and hair (mean ± σ) as well as the percentage of animals suffering from parakeratosis.

Table 3
Effect of dietary zinc on serum, bone and hair zinc content and percent of parakeratotic swine

Day	Group of animals*		
	C ($x \pm \sigma$)	T_1 ($x \pm \sigma$)	T_2 ($x \pm \sigma$)
1 (initial)			
Serum Zn ($\mu g.ml^{-1}$)	0.73 ± 0.14 ←	0.71 ± 0.14	0.73 ± 0.15 →
Hair Zn ($\mu g.g^{-1}$)**		102.10 ± 0.15	
Parakeratosis (%)	0	0	0
28			
Serum Zn ($\mu g.ml^{-1}$)	1.09 ± 0.17†	1.56 ± 0.51†	2.48 ± 1.54†
Parakeratosis (%)	100	0	0
56			
Serum Zn ($\mu g.ml^{-1}$)	0.28 ± 0.07†	1.28 ± 0.14†	1.17 ± 0.14†
Parakeratosis (%)	100	0	0
105			
Serum Zn ($\mu g.ml^{-1}$)	0.39 ± 0.14†	1.34 ± 0.27†	1.26 ± 0.37†
Bone Zn ($\mu g.g^{-1}$)***	83.81 ± 16.88†	111.80 ± 17.20†	103.60 ± 18.67†
Hair Zn ($\mu g.g^{-1}$)**	153.94 ± 5.74†	186.55 ± 14.78†#	173.62 ± 10.13†#
Parakeratosis (%)	0	0	0

* Number of parallels, n = 14 for serum, n = 6 for bone and hair.
** Zn content in hair expressed per gram of dry hair.
*** Zn content in bone expressed per gram of dry, fat-free bone.
† Significant difference ($P < 0.05$) of average Zn levels between either of trial groups and control group.
# Significant difference ($P < 0.05$) of average Zn levels between trial groups.

At the beginning of the trial (d 1) no significant difference of Zn concentration in serum between groups of animals was found. After 28 d of experimental fattening, a considerable increase of Zn concentration in serum was observed in all groups of animals, followed by a remarkable drop and another increase.

After 28, 56 and 105 d of the experimental feeding regime, in control animals a lower ($P < 0.05$) concentration of Zn in serum was found as compared to T_1 and T_2 pigs. No significant difference in serum Zn concentration between T_1 and T_2 was observed. However, after the first month of Zn supplementation a high variability in response of animals to ZnMET was observed. It might have contributed to a transient hyperzincaemia recorded in group T_2.

At the beginning of the experiment, there were no signs of parakeratosis found in any of the groups. After 28 days, however, there was clinical evidence of typical parakeratosis in all control animals. They suffered from reduced appetite and changes on the whole skin (on belly in particular) with intensive itching and red round swellings covered with crusts (1-2 cm in diameter) that tended to become confluent. During subsequent four weeks of the trial (until d 56), the parakeratotic changes persisted (Plate II. Fig. 1 and 2).

In the period from d 56 to d 105 of the trial, the parakeratotic signs on the skin of control pigs disappeared. On the other hand, none of the trial animals have shown signs of parakeratosis throughout the experiment.

The accumulation of Zn in bones of pigs was observed at the end of experiment: the Zn concentration in T_1 and T_2 was higher ($P < 0.05$) than that in C group.

During the trial, an average increase in Zn concentration in swine hair of approximately 70 % was recorded. Upon experimental fattening the Zn concentration was higher ($P < 0.05$) in T_1 and T_2 than in controls. Moreover, a higher ($P < 0.05$) value was found in the T_1 than in T_2 group.

Enzyme catalytical activities

Catalytical activity of enzymes AP, AST, ALT and GGT in serum of pigs (mean ± σ) as monitored throughout the trial is presented in Table 4.

Table 4

Catalytical activity (μkat.l^{-1}) of AP, AST, ALT and GGT in serum

Enzyme	Day	Group of animals*		
		C ($x \pm \sigma$)	T_1 ($x \pm \sigma$)	T_2 ($x \pm \sigma$)
AP	1	2.97 ± 0.49	2.35 ± 0.71	2.73 ± 0.86
	28	1.95 ± 0.44¶	2.80 ± 0.63¶	2.68 ± 0.50¶
	56	0.96 ± 0.34¶	3.00 ± 0.56¶	2.99 ± 0.80¶
	105	0.65 ± 0.30¶	1.54 ± 0.71¶	1.51 ± 0.32¶
AST	1	0.80 ± 0.34	0.85 ± 0.33	0.79 ± 0.15
	28	0.77 ± 0.22¶	0.61 ± 0.11¶	0.70 ± 0.18
	56	0.71 ± 0.19¶	0.53 ± 0.12¶	0.48 ± 0.07¶
	105	0.65 ±0.25	0.82 ± 0.55	0.66 ± 0.25
ALT	1	0.78 ± 0.17	0.77 ± 0.23	0.66 ± 0.15
	28	0.66 ± 0.16	0.74 ± 0.13	0.68 ± 0.08
	56	0.37 ± 0 12¶	0.40 ± 0.13#	0.55 ± 0.08¶#
	105	0.70 ± 0.12	0.74 ± 0.12	0.69 ± 0.13
GGT	1	0.64 ± 0.17	0.65 ± 0.21	0.64 ± 0.29
	28	0.51 ± 0.16	0.51 ± 0.30	0.46 ± 0.16
	56	0.94 ± 0.41¶	0.79 ± 0.23#	0.57 ± 0.30¶#
	105	0.87 ± 0.51¶	0.70 ± 0.18	0.59 ± 0.24¶

* Number of parallels, n = 14.
¶ Significant difference ($P < 0.05$) of average catalytical activity between either of trial groups and the control group.
\# Significant difference ($P < 0.05$) of average catalytical activity between trial group.

At the beginning of the trial there was no statistically significant difference in catalytic activity of any of the enzymes studied among any of the groups.

During the experimental fattening, however, a continuous downward trend of the AP catalytical activity was observed in C group. The respective values were always lower than those in T_1 and T_2. Meanwhile, until d 56 in groups T_1 and T_2 almost the same and constant values were attained. A remarkable decline in catalytical activity was only recorded at the end of experiment.

Considering the AST catalytical activity, no significant differences among C and T_1 and T_2 groups were found either at the beginning or at the end of the experiment. However, during the second month of experimental feeding regime such differences were observed. C group showed a slow but persistent declining trend of the AST activity, but with still higher ($P < 0.05$) values in comparison with trial groups until d 56. In both trial groups similar values and analogous tendencies were observed: a temporary decrease of activity followed by restoration to initial values.

Significant difference ($P < 0.05$) of catalytical activity of ALT between control and either of trial groups was only found on d 56. At this point activity recorded in group T_2 was significantly higher than that in C and T_1 groups. In all experimental groups a similar minimum activity on d 56 was found.

There were no differences detected in GGT catalytical activity of C, T_1 and T_2 groups during the trial, with nearly the same dynamics reaching their minimum on d 28. Lower ($P < 0.05$) activity in T_2 pigs compared to controls was found at the end of the second month of the experiment.

Discussion

During the 30 d period preceding the experiment, all piglets were fed a starter (45.5 mg $Zn.kg^{-1}$ fodder mix) with no extra Zn supplement. This resulted in serum Zn being at the lower limit of the physiological range (0.70-1.50 $g.ml^{-1}$), (Puls 1990) though higher than the values reported for Zn deficiency (0.40-0.50 $g.ml^{-1}$), (Hoekstra et al. 1967). Nevertheless, this relatively low zinc concentration was not accompanied by reduced diet intake or other clinical manifestations of Zn deficiency. This feeding regime was aimed at exhaustion of the Zn depots (liver, bones and other tissues) in all groups of pigs in order to detect nutritional efficiency, if any, of different amounts and sources of dietary Zn in trial groups.

It should be noted that during the second month of the experiment clinical signs of typical parakeratosis (itching, discoloured skin) were evident in all control animals but in none of trial animals. During the whole trial (105 d) performance data in all animals were very good. Clinical signs of parakeratosis were accompanied by deteriorated production data in the controls e.g. by reduced food consumption and lower body mass gain compared to T_1 and T_2 pigs. In the period from d 56 to d 105 parakeratotic changes on the skin of control animals disappeared but itching and poor production continued. Disappearance of pathological parakeratotic changes may be attributed first to a decline of growth rate and body mass gain (diminished protein synthesis rate) in this period when feed began to be predominantly converted into fats, and secondly by increased food intake sufficient to cover the basic metabolic needs for zinc.

The finding of transient increase of Zn concentration in blood serum of control pigs was somewhat surprising at a time when parakeratotic changes first occurred. They were culminating at the time when the lowest serum Zn concentration was measured on d 56 (0.28 $\mu g.ml^{-1}$). This coincided with the substitution of starter with grower (containing lower Zn amount) and reduced food intake.

Our results are in accordance with those of Eder et al. (1996) who reported on decreased serum Zn concentration by 70% as a consequence of dietary Zn deficiency. Groups T_1 and T_2 showed significantly higher Zn concentrations in serum than control group; moreover, neither symptoms of parakeratosis nor any other health disturbance was noticed. This is in accordance with the fact that the serum Zn stabilized at the upper physiological limit. Considerable drop of Zn concentration in serum during the second month of the trial (d 28-56) may be explained by both intensive body mass gain and augmented synthesis of metallothionein. Compared to the first month of the experiment, daily body mass gain recorded during the second month for groups T_1 and T_2 was increased on average by 16 and 11 %, respectively. This resulted in increased metabolic need for zinc. On the other hand, the abundant intake of zinc in both groups probably induced the synthesis of metallothionein (Kelly et al. 1996; Richards 1989) that bound the excess of zinc and stored it in the liver, bones, and pancreas. In addition, successive decrease of Zn concentration (added as ZnMET) in starter, grower and finisher diets may have participated in the decreasing serum zinc concentration in T_2 group.

Zn concentrations in serum speak in favour of higher nutritional availability and efficiency of organic zinc during the whole trial, particularly in first 2-3 months of life when growth and body mass gain (predominantly protein synthesis) is very intensive. Although the concentration of Zn supplemented to trial animals in the form of metalo-organic chelate was half the amount of the inorganic salt it was enough to cover the metabolic requirements and maintain good health and performance of animals. All these facts confirm the better bioavailability of Zn from ZnMET than from $ZnSO_4$. This is in accordance with the results of Hill et al. (1986), Swinkels et al. (1991) and Wedekind et al. (1992, 1994). Better bioavailability of Zn from chelate presumably results also in markedly lower amount of Zn eliminated by faeces and in reduced contamination of environment. Therefore, as a diet additive, ZnMET is preferred over $ZnSO_4$ from both nutritional and ecological point of view.

Our results on Zn amount found in coccygeal vertebrae as well as the deposition sequence of Zn from different sources in bones are in agreement with findings of Wedekind et al. (1994). Our values, however, are lower than those of Hoekstra et al. (1967) reported for adult pigs.

Remarkable nutritional influence of different sources of zinc on porcine hair was demonstrated first by a sharp increase in Zn content in hair which was on average 70 %, and also by higher concentration of zinc in T_1 and T_2 groups than in control group. However, significantly greater amount of inorganic than of organic zinc was deposited in hair. At the end of experiment we found Zn concentrations in hair exceeding considerably those reporterd for adult pigs by Hoekstra et al. (1967).

Feed efficiency of Zn supplemented to fodder mixes was also documented through superior feeding variables found in T_1 and T_2 groups compared to control. However, better feeding variables observed in T_2 compared to T_1 were not significant. All nutritional indicators evaluated in our experiment are in full accordance with the similar results obtained by Wedekind et al. (1994).

Catalytical activity of AP in serum is a valuable diagnostic tool. Apart from other clinical and biochemical evidence, the increased activity of AP indicates pathological processes in bones, liver and bile ducts, whereas its lower activity points to disturbances in synthesis of AP which may result in decreased deposition of minerals in skeleton. Since Zn is a part of AP molecule, it is essential both for its synthesis and catalytical activity. Animals fed inadequate Zn amount may obtain insufficient Zn amount to cover their basic metabolic needs with resulting low AP activity in serum. This was apparently the case in our control animals having significantly lower catalytical activity of AP than T_1 and T_2 and decreasing throughout the experiment (see Table 4). On the other hand, in T_1 and T_2 pigs, high and practically constant values were recorded. Irrespectively of the organic or inorganic zinc supplied almost the same Zn concentrations were found in trial groups. These results support the idea of a direct correlation between AP catalytical activity in serum and diet Zn intake. Moreover, a linear correlation (correlation coefficient of 0.90) was found between AP catalytical activity and Zn concentration in serum. Our results are consistent with data of Luecke et al. (1958) and Hoefer et al. (1958, 1960) who investigated the influence of Zn-deficient feeding regime accompanied by metabolic disorder of parakeratosis on the concentration of Zn and catalytical activity of AP. However, our experimental data for the AP activity in serum of both trial groups were beyond those reported by Imlah and McTaggart (1977) and Forenbacher (1993) for healthy pigs. Just the opposite is true for the controls (Odink et al. 1990).

Lower catalytical activity of AP in serum of control pigs pointed to subclinical disturbances in bones confirmed by lower ($P < 0.05$) concentration of Zn than in the groups

T_1 and T_2. Zn deficiency in controls was also reflected in lower ($P < 0.05$) level of Zn in hair compared to trial pigs.

Aminotransferases (AST, ALT) and GGT take part in metabolism of proteins, and they indicate pathological processes in liver and, to a lesser extent, serve to evaluate the muscular activity (AST).

Throughout the trial the catalytical activity of AST was high in controls, on d 28 and 56 even surpassing ($P < 0.05$) that of T_1 and T_2 pigs. Interestingly, at this time control pigs have shown parakeratotic changes. Their AST activity had a slight continuous downward trend. At the end of experiment, when parakeratotic changes on skin ceased, no difference in AST between C versus T_1 and T_2 pigs was found. Hence it seems that in the course of development of pathological changes on the skin during intensive body mass gain (protein synthesis prevailing in skeletal muscles) AST activity raises.

Our data on AST catalytical activity in swine serum were higher than those reported as physiological by Imlah and McTaggart (1977), Žurić and Stanković (1991) and Forenbacher (1993) but remarkably lower than those found by Odink et al. (1990) in healthy pigs.

Catalytical activity of ALT in serum was almost the same in control and in trial groups throughout the experiment, indicating that neither deficiency nor addition of zinc affected the activity of this enzyme. No chronic dietary hepatosis was thus diagnosed. Our data for catalytical activity of ALT are comparable with those of Odink et al. (1990) but markedly higher than those of Žurić and Stanković (1991) and Forenbacher (1993).

No differences were found in the catalytical activity of GGT on d 1 and 28 among C, T_1 and T_2 pigs. However, on d 56 and 105 detected higher GGT activity in C pigs was indicative of a possible liver malfunction in these animals caused by a long-term deficiency of zinc in the diet. Moreover, a linear correlation of GGT catalytical activity and serum Zn level with a correlation coefficient of -0.72 was established. Our values for the GGT activity in control animals until d 28 and in trial animals throughout the experiment were comparable with those found by Žurić and Stanković (1991) and Forenbacher (1993) for healthy pigs, but somewhat higher in regard to the controls in the period from d 28 to the end of experiment. However, our values were remarkably lower than those of Odink et al. (1990).

Some differences in our data for catalytical activities of AP, AST, ALT and GGT compared to those reported in literature might be attributed to different experimental conditions, possibly incubation temperature. Because our values were measured at 30 °C they were expectedly higher than those reported by Imlah and McTaggart (1977), Žurić and Stanković (1991) and Forenbacher (1993) for 24 °C, comparable to those measured by Luecke et al. (1958) and Hoefer et al. (1958; 1960) at 30 °C, and lower than those of Odink et al. (1990) obtained at 37 °C.

Vliv příjmu anorganického a organického zinku na jeho koncentraci v krevním séru, v kostech, ve štětinách a vliv na katalytickou aktivitu některých sérových enzymů u prasat

V práci je srovnáván vliv příjmu zinku z anorganického a organického zdroje u prasat trpících jeho deficitem. Třicet dnů před pokusem byl zjištěn u vyšetřovaných prasat (německá landrase × pietrain × bílé ušlechtilé × švédská landrase) nedostatek zinku. Prasata byla rozdělena do tří skupin po 14 kusech. Během experimentálního krmného období (105 dnů) nebyla kontrolní skupina krmena vyšším obsahem zinku, zatímco zbývajícím dvěma pokusným skupinám byl podáván zinek ve formě anorganické ($ZnSO_4$) a ve formě metaloorganického

chelátu (methionát zinku). Jednotlivé vzorky obsahovaly průměrně 84,3 mg Zn/kg^{-1} a 40,9 mg Zn/kg^{-1} v krmivu.

Hodnocení výživného účinku zinku bylo založeno na jeho koncentraci v krevním séru, v kostech a ve štětinách. Dále byl studován vliv zinku na katalytickou aktivitu alkalické fosfatázy, aspartát aminotransferázy, alanin aminotransferázy a gama-glutamyl transferázy v séru.

Snížená katalytická aktivita aspartát aminotransferázy a alanin aminotransferázy byla zaznamenána současně s parakeratózou kůže u kontrolních zvířat a nevztahovala se k množství zinku v séru. V protikladu k těmto nálezům byla zaznamenána lineární korelace mezi katalytickou aktivitou alkalické fosfatázy a gama-glutamyl transferázy a koncentrace zinku v séru se pohybovala od 0,90 do -0,72.

Výživná hodnota zkrmovaného zinku byla vyšší ($P < 0,05$) v séru, v kostech, ve štětinách, v hepatobiliárním traktu a ve srovnání s kontrolní skupinou byly získány příznivé výsledky.

U žádného pokusného zvířete nebyly zjištěny jiné zdravotní problémy. U kontrolních zvířat propukla progresívní parakeratóza, která pokračovala ještě do druhého měsíce pokusu. Tím byly nepříznivě ovlivněny získané údaje postižených zvířat.

Pokusem jsme dokázali, že zinek byl lépe využit z chelátu než z anorganických solí, zejména v období rychlého růstu. Vzhledem k lepšímu využití u prasat a z hlediska ekologického by měla být dána přednost krmivu obohacenému $ZNSO_4$.

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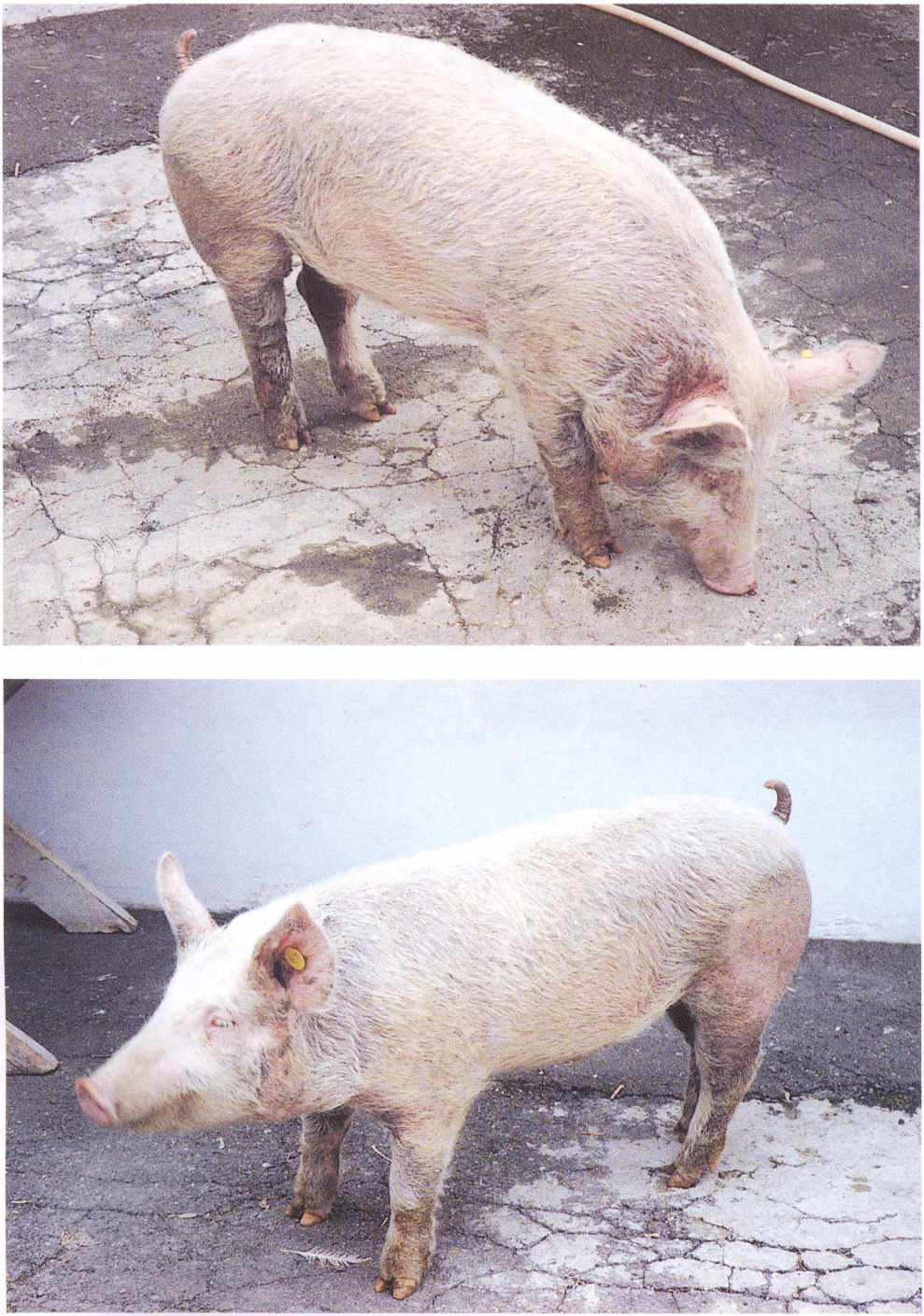

Fig. 1. and 2. Typical parakeratotic changes on the skin of head, neck, front and back legs.