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EFFECTS OF SUPPLEMENTATION OF ORGANIC-BOUND TRACE ELEMENTS ON BLOOD AND TISSUES - MICROMINERAL PROFILE AND IMMUNE PARAMETERS OF PIGLETS

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An 8-week experiment was carried out on piglets to observe the effects of organic trace element supplementation (Piglet Booster Alltech, Inc.) on the micromineral profile of blood serum, immunological parameters, body weight (before and after weaning), and tissue concentrations of Zn, Fe, Cu, Mn, and Se. Forty piglets from 4 sows were divided into 2 groups (control – 19 piglets, experimental – 21). Organic trace element supplementation affected positively the body weight of the experimental animals. The metabolic activity of phagocytes and polyclonal activation of lymphocytes were significantly increased ($p<0.05 - 0.01$) in the second phase of the experiment. Significant increases in Fe, Cu, and Se in the blood serum ($p<0.05 - 0.001$) and Zn, Se, and Mn in the heart muscle ($p<0.05$) were recorded. Increased concentrations of minerals were observed in the heart (Fe), liver (Zn, Fe, and Se), kidneys (Zn, Fe, Se, and Mn), and loin muscles (Fe, Se, and Mn).

Key words: piglets, organic trace elements, micromineral profile, immune parameters

INTRODUCTION

The trace elements iron, copper, zinc, selenium, and manganese fulfil important structural, physiological, catalytic, and regulatory functions in animal bodies (Underwood and Suttle, 1999). They act particularly as components or activators of enzymes, co-enzymes, and hormonal mechanisms of animal bodies, affecting in this way the function of cardiovascular, central nervous, immune, and reproductive systems (Power and Hogan, 2000).

Concentrations of trace elements in animal tissues depend mostly on their content in the feed, gastrointestinal absorption, and homeostatic control mechanisms of the body. Unsuitable proportions of trace elements in the rations result in decreased concentrations in body tissues. On the contrary, excess uptake of trace elements leads to their deposition in tissues and excretion before or after absorption (Richards and Close, 2001).

Supplementation of rations with organic forms of minerals bound to amino acids or short-chain peptides is one of the methods that can be used to increase the availability of trace elements to piglets. The organic-bound trace elements are more resistant to reactions with other chemical compounds during digestion and more soluble. Therefore, they are more easily absorbed and integrated into biological reactions and body structures (Richards and Close, 2001).

The aim of our experiment was to evaluate the effect of short-term supplementation of organic-bound trace elements on the organism of the piglets before and after weaning. The effect was evaluated on the basis of body weight of piglets, reactivity of cells of the immune system, micromineral serum profile and concentration of Zn, Fe, Cu, Mn, and Se in the heart, liver, kidneys, and loin muscles of the piglets.

MATERIALS AND METHODS

The animals used: Piglets from 4 litters of the same age were divided to 4 groups: experimental (n=21) and control (n=19).

Experimental design: The experiment consisted of two phases: the first phase included the period of milk feeding (suckling period) and the second phase the period after weaning (weaning at 6 weeks of age). The suckling piglets (1^{st} phase) were fed ad libitum a mixed feed OŠ-01 (Tajba, a.s., Čana) while the weanlings were supplied with a dry mixed feed OŠ-02 (Agronákup s.r.o., Trebišov) and OŠ-03 (Tajba, a.s., Čana, Tab. 1), following a restricted regimen. The rations supplied to the experimental group were supplemented with organic-bound trace elements (Zn, Fe, Cu, Mn, and Se proteinates) in the form of a commercial preparation (Piglet Booster, Alltech Inc., Table 1) at a dose of 1g preparation per kg of the ration, starting from the 31^{st} day of age (beginning of the experiment).

Table 1. The micromineral content of OŠ-01, OŠ-02, OŠ-03 and the Piglet Booster

mg/kg	Fe	Cu	Zn	Mn	Se
OŠ-01	211.37	20.56	118.44	70.07	0.080
OŠ-02	183.55	18.16	118.65	46.35	0.076
OŠ-03	180.26	22.61	110.58	39.20	0.062
Piglet Booster	19605	14253	18439	9600	0.147

The blood was sampled from the eye sinus (Kováč *et al*., 1990) on the 31^{st} day of age (sampling 0) then at weekly intervals until the 45^{th} day of age. Later samplings were at 66 and 87 days of age. The experiment lasted 8 weeks and took place at the IInd Internal clinic of the UVL in Košice.

The body weight of the piglets was determined at weekly intervals starting from day 31. At the end of the experiment (day 87 of age), altogether 8 animals (4 from each group) were slaughtered and samples of heart, liver, kidneys and loin muscles were taken and stored at $-18\ ^{o}C$ up to the analysis.

Determination of concentrations of trace elements in tissues and blood serum: Tissue samples were subjected to wet mineralization in a microwave oven

(MLS 1200). Tissue and serum concentrations of Cu, Fe, and Zn were determined by atomic absorption spectrometry (AAS) using a Perkin Elmer AAnalyst 100 apparatus and the flame method. Concentrations of Se and Mn were measured by the non--flame AAS method with 4100 ZL equipment.

Immunological tests: Tests for the analysis of immunological parameters were selected to evaluate the functional activity of phagocytic cells (the metabolic activity of phagocytes) by the iodo-nitro-tetrazolium reductase test and of lymphocytes (the activation of lymphocytes with phytohaemagglutinin) by the leukocyte migration-inhibition test. Leukocytes were isolated from the peripheral blood according to Karlson and Kaneko (1973).

a) Iodo-nitro-tetrazolium reductase test (INT). A quantitative evaluation of the tetrazolium-reductase activity of leukocytes for the evaluation of the metabolic activity (MA) of phagocytes during phagocytosis was carried out according to the method described earlier (Revajová et al., 2001). The results are expressed in the form of an index of metabolic activity (IMA) based on the ratio of the mean optical density of leukocyte suspensions with (n = 3) and without starch (n = 3).

b) Leucocyte migration-inhibition assay (LMIA). LMIA in agarose was used to analyse the reaction capacity of lymphocytes to mitogenic activation and was carried out according to the method described earlier (Revajová *et al*., 2001). The migration index (MI) was determined as a ratio of the mean of migration areas of leukocytes with and without mitogen (phytohaemagglutinin, PHA, Sigma, Germany).

Statistical processing of results: The results obtained were processed by Student's t-test.

RESULTS

The mean body weight of control piglets was higher in the period from delivery up to day 66 of age in comparison with the experimental group. Despite that, during the experiment (days 31-87 of age), the mean weight of the experimental group increased by 18.03 kg, while that of the control group increased only by 15.91 kg (Table 2).

Table 2. The average live weight of the piglets (kg) from the control and experimental groups

	Age of piglets (days)									
Weighing	Birth	31.	38.	45.	52.	59.	66.	73.	80.	87.
Experiment	1.55 ±0.27	8.30 ±1.32	9.99 ±1.76	11.80 ±1.94	13.67 ±2.57	15.32 ±2.60	18.24 ±3.54	20.76 ±3.93	23.54 ±4.43	26.33 ±4.76
Control	1.67 ±0.29	9.12 ±1.31	11.15 ±1.86	12.65 ±1.83	14.37 ±2.66	16.13 ±2.89	18.78 ±4.29	20.31 ±5.29	22.89 ±4.98	25.03 ±4.81

A significant increase in the mean value of IMA of phagocytes ($p<0.05$ – 0.01; Table 3) was observed in 45-day old piglets (14th day of supplementation) while a significant decrease in the mean value of MI was recorded in 87-day old piglets (56th day of supplementation) which suggested an increase in polyclonal activation of lymphocytes by mitogen ($p<0.05$, Table 4).

Table 3. Mean indices of metabolic activity of phagocytes in the peripheral blood of the pigs (INT-Iodonitrotetrazolium test)

Days of sampling	31.	38.	45.	66.	87.
Experiment	2.83±0.17	2.77±0.18	2.65±0.15 ^{a}	2.55±0.12 ^{b}	2.44±0.13 ^{b}
Control	2.75±0.19	2.68±0.13	2.55±0.13	2.41±0.14	2.31±0.13

^{a} $p<0.05$; ^{b} $p<0.001$

Table 4. Mean values for migration-inhibition indices of peripheral blood leukocytes from the pigs (LMIA –Leukocyte Migration - Inhibition Assay)

Days of sampling	31.	38.	45.	66.	87.
Experiment	0.74±0.09	0.69±0.07	0.63±0.11	0.60±0.10	0.54±0.08
Control	0.74±0.09	0.72±0.09	0.61±0.16	0.62±0.06	0.60±0.08^{a}

^{a} $p<0.05$

A significant increase in Fe ($p<0.05$), Cu ($p<0.05$-0.001), and Se ($p<0.05$-0.01; Table 5) was observed in the blood serum of the experimental animals. Concentrations of Mn were higher in the experimental piglets compared to the controls on day 66 of age. No significant difference in mean levels of zinc were observed between the experimental and control animals.

The highest mean tissue concentrations of Zn, Cu, Fe and Mn were observed in the liver and the highest concentration of Se was recorded in the kidneys of both groups of animals. Supplementation of organic-bound trace elements increased significantly the level of Zn, Se, and Mn in the heart muscle ($p<0.05$; Table 6). The experimental group of weanlings exhibited an insignificant increase in mean values for Zn in the liver and kidneys, Fe in the heart muscle, liver, kidneys, and loin muscles, Se in the liver, kidneys and loin muscles, and Mn in the kidneys and loin muscles. On the contrary, the mean values of Cu were higher or identical (kidneys) in all tissues of control animals compared with the experimental ones. The levels of Zn in the loin muscles and those of Mn in the liver were also higher in the control piglets.

Table 5. The concentration of trace elements in the blood serum of the pigs (mol/l) from both groups

	Days of sampling	31.	38.	45.	66.	87.
Zn	Experiment	11.97±1.92	11.67±1.61	11.35±1.50	11.32±1.10	11.54±1.22
	Control	11.50±2.45	11.10±1.80	11.38±1.68	11.91±1.86	11.38±1.61
Fe	Experiment	16.20±2.99	19.40±4.35	21.85±6.61[a]	20.08±4.62	22.34±6.11
	Control	19.13±5.11[a]	18.26±4.10	17.76±3.80	19.27±4.06	19.55±4.61
Cu	Experiment	21.92±2.52[b]	24.28±4.20[c]	23.12±2.41[a]	22.87±1.77[a]	22.89±1.96[a]
	Control	18.67±4.23	18.64±4.58	19.55±5.17	20.13±4.52	20.46±3.40
Se	Experiment	0.294±0.09	0.320±0.09	0.330±0.09	0.332±0.08[a]	0.333±0.08[b]
	Control	0.296±0.06	0.301±0.06	0.302±0.05	0.264±0.08	0.261±0.07
Mn	Experiment	0.399±0.07	0.409±0.08	0.416±0.08	0.418±0.08	0.422±0.09
	Control	0.425±0.06	0.427±0.06	0.426±0.07	0.389±0.10	0.396±0.10

[a] $p<0.05$; [b] $p<0.01$; [c] $p<0.001$

Table 6. The concentration of trace elements in the tissues of 87 day old pigs (mg/kg) from both groups

		Heart	Liver	Kidney	Loin muscles
Zn	Experiment	18.35±0.10[a]	36.67±7.65	20.63±2.26	11.23±0.61
	Control	11.44±2.30	23.75±0.88	17.18±3.48	12.21±1.54
Cu	Experiment	2.26±0.26	6.43±0.51	4.84±0.52	0.56±0.22
	Control	2.80±0.04	7.15±0.56	4.84±0.28	0.62±0.15
Fe	Experiment	33.11±3.41	171.32±33.3	36.81±4.26	7.16±1.04
	Control	31.94±1.23	114.93±3.83	29.40±3.77	6.11±0.52
Se	Experiment	0.077±0.005[a]	0.239±0.042	0.304±0.035	0.099±0.02
	Control	0.055±0.012	0.205±0.034	0.229±0.046	0.088±0.01
Mn	Experiment	0.329±0.037[a]	4.948±0.404	0.922±0.203	0.102±0.025
	Control	0.242±0.002	6.030±0.440	0.840±0.02	0.089±0.007

[a] $p<0.05$

DISCUSSION

The liver plays a key role in the biochemical and metabolic processes involving trace elements. Our experiment showed that the highest concentrations of Zn, Cu, Fe, and Mn were detected in this organ in both observed groups. Contrary to that the highest Se levels were reached in the kidneys, which play the most important role in excretion of Se in monogastric animals (Osweiler, 1996). Short-term supplementation of trace element proteinates resulted in an approximately 1.5-fold increase in liver Zn and Fe and approximately a 1.3-fold increase in Se concentration in kidneys. The supplementation with trace elements led to increased concentrations of Fe, Cu, and Se in the blood serum too. The significant increase in serum concentration of trace elements following supplementation of rations with organic-bound forms is in agreement with the experiment of Kováč *et al.* (2000), carried out in piglets in the pre-fattening period.

The mean daily weight gain of piglets fed rations supplemented with organic-bound trace elements reached 321 g in the period of supplementation (from day 31 till 87 of age) compared to the 284 g in the control piglets. The mean daily weight gain in individual weeks (229 g, 271 g, 267 g, 235 g, 390 g, 387 g, 397 g, 399 g) was within the range of 226 - 417 g that was observed by Buchová *et al*. (2000). The difference between the mean weights of the investigated groups over the 8-week period was 2.12 g in favour of the experimental group.

The animals in the experimental group showed increased levels of parameters of metabolic activity of phagocytes and mitogenic activation of lymphocytes, which can be closely related to the role of individual trace elements in the body. Fe affects the intracellular killing of micro-organisms by polymorphonuclear leukocytes and the response of lymphocytes to mitogens and antigens (Mac Dougal *et al.,* 1975). Fe in combination with vitamin E increases the percentage of rosetting T-lymphocytes and the index of metabolic activity of phagocytes (Mudron, 1995). Copper is an important component of intermediary metabolism and its relationship to the immune system is mediated by superoxide dismutase and its functions in the microbicidal system of phagocytes (Miller *et al*., 1979). Cu also affects the weight of the thymus, which is related to the number of T lymphocytes and the effect on their functions (Lukasewycz *et al.,* 1985, Flynn, 1984). Mn, as a part of superoxide dismutase, is an element important for the enzymatic antioxidative system (Matés, 1999). A deficit of zinc is manifested particularly by involution of the thymus, which is reflected in a decreased number of T lymphocytes and deficiencies in their response to mitogens (Ades *et al.,* 1980). The effect of selenium has been described in association with vitamin E. Selenium acts as an antioxidant and protects cellular membrane lipids of lymphocytes against peroxidation preserving their integrity (Wuryastuti *et al.,* 1993). It was proved that it also affects phagocytic functions with respect to uptake and killing of micro-organisms and oxidative metabolism of neutrophils (Dimitrov *et al.,* 1984).

We may conclude that short-term supplementation of piglets with organic-bound trace elements in the period before and after weaning caused slight to significant increase in the levels of the investigated trace elements in the blood serum and tissues of piglets which resulted in increased weight gains during the experiment. The results of immunological tests point to an increase in the natural resistance of piglets from the experimental group.

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SUPLEMENTACIJA ORGANSKI VEZANIM MIKROELEMENTIMA, MIKROMINERALNI PROFIL KRVI I TKIVA I IMUNOLOŠKI PARAMETRI PRASADI

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SADRŽAJ

U osmonedeljnom eksperimentu testiran je uticaj suplementacije organski vezanih mikroelemenata na mikromineralni profil krvnog seruma, imunološke parametre, telesnu masu (pre i posle odbijanja) i koncentraciju Zn, Fe, Cu, Mn, Se u tkivima prasadi. Četrdeset prasadi poreklom od 4 krmače je bilo podeljeno u 2 grupe (kontrolna grupa – 19, eksperimentalna grupa – 21) i registrovan je pozitivan uticaj suplementacije organski vezanih mikroelemenata na telesnu masu.

Metabolička aktivnost fagocita i poliklonalna aktivacija limfocita su se značajno povećale u drugom delu eksperimenta ($p<0,05$; $p<0,01$). Takođe je zabeleženo značajno povećanje koncentracije Fe, Cu, Se u krvnom serumu ($p<0,05$; $p<0,01$; $p<0,001$) i koncentracije Zn, Se, Mn u srcu ($p<0,05$).