RESEARCH ON THE USE OF INORGANIC AND ORGANIC COPPER (COPPER SULPHATE AND MINITREX-CU WITH METHIONINE) IN PIGLET DIETS, WITH THE PURPOSE TO REDUCE EXCRETED COPPER ACCUMULATION IN THE SOIL

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Abstract

The effect of the organic copper (Minitrex-Cu with methionine) and of the inorganic copper (Cu SO_4+5H_2O) was studied with the purpose to reduce the adverse effects of the copper from excretion on the soil. A group of 96 hybrid Great White x Landrace piglets, weaned at 32 days, with an average initial weight of 8.27 kg, were used in a three-week experiment. The animals were assigned to 6 groups: three groups treated with copper sulphate (L1S - 50 ppm Cu; L2S-150 ppm Cu; L3S-250 ppm Cu) and the other three groups were treated with Minitrex-Cub (L1M -50 ppm Cu; L2M-150 ppm Cu; L3M-250 ppm Cu). The formulation of diets was identical for all groups, the difference between them being the amount of copper (50;15 and; 250 ppm). The serum copper and iron concentration were determined on blood samples. The animals were weighed in the beginning and end of the trial and the feed intake was recorded. The antibiotic effect of the copper was monitored by recording daily the health state of the animals. The effect of the copper source on the copper concentration in the faeces was determined by analytical determinations. Cu and Fe were determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES). The conclusions of the experiment show that the use of the dietary cooper chelates decreased the concentration o copper in pig manure, without affecting animal performance

Key words: **copper, chelate, piglets, feed additives**

INTRODUCTION

Because of the common practice of using an excess amount of nutrients in animal feeding (more than animal requirements), excess which is eliminated mainly through excreta, policies were developed lately to minimize the potentially polluting inputs for the ecosystems neighbouring the animal farms. (J. M. Burkholder; David A. Dickey, 2007.). One of the contaminants which pollute the environment is the copper, mineral used as growth promoter and as alternative to antibiotics. Thus, significant amounts of ingested copper are eliminated in the excreta of the farm animals. The copper which is assimilated in the soil has toxic effects on the plants and soil microorganisms, which is a threat to the environment in the areas neighbouring intensive animal farming (Kornegay Versteges et al. 2001).

The great difficulty of these determinations is due mainly to the fact that the recommendations must integrate closely into the complex of factors influencing the process of nutrition: complexity of the diet, interactions with other feeding principles, energy level of the diet, stress factors (heat stress, diseases, crowding etc.) and the source of copper. These mentioned factors modify the intrinsic value and disperse animal performances. In practice, the copper supplements to piglet diets are added according to the recommendations of the premix manufacturers (concentrates, mixtures). Most times, these recommendations are not the result of scientific data taking into account the complex of factors mentioned above and the supplements increase 2, 3 times the requirements determined by scientific investigations. In 2004, it was estimated that in the United Kingdom 30% of the copper inputs on the agricultural land come from farm animals manure, the precise figure being 540 tons of copper (Nicholson and Chambers, 2006). The inorganic compounds currently used as feed additives in animal nutrition must undergo hydrolysis in the gastric fluid. This hydrolysis is influenced by several factors such as: area where absorption takes place and the pH value of the digestive tract environment. Once the hydrolysis is accomplished, the copper ion becomes very sensitive to any oxidoreduction reaction that may appear in the reaction environment and become chemically available to be chelated by any ligand existing in the stomach fluid before entering the duodenum.

This complex of factors and biochemical reactions make the inorganic salts of the minerals release species of ions into the digestive tract, which are not suitable for the digestion processes and which, therefore, decrease much the bioavailability of the minerals.Because of the low bioavailability, most trace elements from a salt are eliminated into the excreta.

For instance, one of the heavy metals commonly used as growth promoter, copper, after ingestion is absorbed in a proportion of only 25-30% in the small intestine, the balance being eliminated through the excreta. The literature reflects the lack of experimental data which to fundament different systems of recommendations of the sources and levels of feed additives. J. Pallauf – in 1996 at the 47th Meeting of the European Association for Animal Production (Lillehamer), highlighted the large differences between the mineral recommendations for piglets released by the different scientific bodies.

The administration of higher amounts of copper to piglets will increase the amount of excreted copper (manure). The excess of copper, assimilated in the soil has toxic effects on the plants and soil microorganisms and creates an environmental problem in the areas neighbouring intensive pig farming sites (Kornegay Versteges et al. 2001).

Feeding, and not only, is working to reduce the amount of excreted copper. Among the approached solutions are the balancing of the requirement/supplementation ratio and the use of supplements with a higher bioavailability (Hill et al. 2007). Given the unwanted effects of the copper and the disturbance of the physical and chemical properties of the soil (the polluted soils have a lower percentage of aggregation and a lower hydric stability), it results that the excess of copper produces soil erosion and soil compaction. It is therefore necessary to identify sources of copper with a higher bioavailability, which minimalize the inputs of pollutants in the ecosystems neighbouring the pig farms. The physiological requirements for copper are known, but it is not yet exactly known how much copper has to be added besides the amount already existing in the forages in order to obtain a good performance in terms of quantity, quality, economy and soil protection

MATERIAL AND METHOD

The study was conducted in the experimental farm of the National Research-Development Institute for Animal Biology and Nutrition (IBNA Balotesti) within Project POSDRU/89/1.5/S/63258 „*Post-doctoral school for zootechnical biodiversity and feed biotechnologies based on eco-economy and bio-economy necessary for ecosanogenesis*”.The experiment used 96 hybrid Great White × Landrace piglets, weaned at 32 days, with an average initial weight of 8.27 kg, on a period of three weeks.

The animals were assigned to 6 groups: three groups treated with an inorganic source of copper (copper sulphate ($CuSO_4+5H_2O$), each group being treated with a different amount of dietary copper: L1S - 50 ppm Cu; L2S-150 ppm Cu; L3S - 250 ppm Cu; the other three groups were treated with an organic source of copper (Minitrex-Cu with methionine), each group being also treated with a different amount of dietary copper: L1M -50 ppm Cu; L2M - 150 ppm Cu; L3M - 250 ppm Cu, as shown in the experimental design (Table 1).

Table 1

Experimental design

Group	**L1S**	**L2S**	**L3S**	**L1M**	**L2M**	**L3M**
Number of animals	16	16	16	16	16	16
Copper sulphate (ppm Cu)	50	150	250	-	-	-
Minitrex-Cu (ppm Cu)	-	-	-	50	150	250
Duration, days	21	21	21	21	21	21

Feeding was *ad-libitum*, using the same compound feed formulation (NC-01) adequate to this category of age, with six variants which differed only by the source and amount of dietary copper.

Compound feed formulation NC-01: corn - 50.38%; soybean meal - 15.0%; milk replacer -20.0%; fish meal- 4.0%; soybean oil -5.00%; choline premix -0.22; lysine - 0.60%; methionine - 0.40%; dicalcium phosphate - 2.40%;

calcium carbonate - 0.50%; salt - 0.50% premix protein-vitamin-mineral concentrate P_{1+2} - 1%.

Quality indices of NC-01: crude protein 21.32; lysine 1.5; methionine + cystine 1.06; calcium -1.16; phosphorus - 0.98; metabolisable energy MJ/kg CF - 14.71. Copper was included in the premix P1+2 in amount of 1%. Table 2 shows the formulation of P_{1+2} protein-vitamin-mineral concentrate. The piglets were monitored to determine whether the different amounts of organic copper can preserve the growth performance while decreasing the amount of excreted copper.

The blood concentration of copper and iron was determined. In the beginning and end of the experiment, blood samples (15-20 ml) were collected from 6 piglets/group, from the jugular vein, in plastic tubes. After decantation the serum was centrifuged in a Beckman centrifuge at 2500 rpm for 10 minutes. Part of the serum was diluted 1/5 ml in potassium chloride, concentration 1.34 M, and analysed for copper and iron determination.

Evaluation of the copper concentration in the excreta. Every week, average samples of faeces were collected and then formed for each of the six groups. About 20% of the total amount of manure was collected from the 96 piglets assigned to 6 groups housed in 36 pens, as follows:

-18 samples from 18 pens/3 groups of piglets treated with copper sulphate;

- 18 samples from 18 pens/3 groups of piglets treated with Cu-Minitrex with methionine).

The samples of faeces were kept in plastic containers, weighed on an analytical scale Sartorius (Gottingen, Germany), and dried at 60^0C in BMT stove model ECOCEL Blueline Comfort.Disaggregation was done with a BERGHOF microwave digester with remote temperature determination; model Speedwave MW-2 Comfort (Eningen, Germany).The distilled water was obtained with the Milli-Q Ultrapure Water Purification System Millipore (Billerica, USA).Class A glassware was used for transfer, dilution and storage.Inductively coupled plasma atomic emission spectroscopy (ICP-AES) was used to *determine the blood and faeces concentration of copper.* This is a highly accurate method to determine trace metals from different types of samples. The determinations were done on a spectrometer (Spectra-Span VI) whose working principle is the inductively coupled plasma atomic emission spectroscopy (ICP-AES).

The stage of instrumental analysys involves the production of the analysis spectrum for copper and iron at very high temperature (2000^0C). The optical system allows the selection of a spectral line of a specific wavelength whose intensity is measured by a photomultiplier. The signal generated by the photomultiplier is amplified, converted digitally and stored in the memory. The adjustment of the optimal analytical parameters by digital calibration (selection of the element to be analysed, setting the wavelength, setting the measuring units, the maximal and minimal limit of detection, sensitiveness, and selection of the diffraction system – PMTV) ensures the accuracy and reproducibility of the copper and iron determinations.

The analytical parameters for copper and iron determination were selected function of the amplitude of the spectrum peak (Table 2).

Table 2

Analytical parameters used to determine copper by ICP-AES

Element	μ. (nm)	Physical-chemical state	Plasma position	Detection limit DL	Precision RSD, %
Cu 2+	224.700	ion	0	0.002	0.02
Fe	259.94	ion	0	0.009	0.03

Animal performance parameters – bodyweight evolution was monitored by the individual weighing of the piglets at the beginning and end of the experiment; the average daily feed intake, the average daily gain and the feed conversion ration were monitored throughout the experiment.

Two samples were collected from each batch of compound feed, for each variant of formulation, and assayed in the laboratory for Cu and Fe concentration. The gross chemical composition determinations (protein, fat, fibre, ash, energy) was determined in the laboratory of the manufacturer (Pilot station of IBNA-Baloteşti).The *frequency of enterocolitis* was monitored using health information records kept on a daily basis.

RESULTS AND DISSCUSIONS

In the *groups treated with copper sulphate,* the level of serum copper concentration increased with the dietary level, but the increase was not linear. Serum copper was similar for groups L3S (250 ppm Cu) and L2S (150 ppm Cu), showing that above this concentration the dietary Cu has no more effects. This shows that in group L3S, the copper supplementation was excessive, excess which tends to be eliminated (Figure 1).

Significant differences were noticed in the groups treated with Minitrex-Cu compared to the groups treated with $CuSO_4$+$5H_2O$ ($P \geq 0.05$), however without exceeding the normal limits. These results were also confirmed by the reports of Stansbury, W. F., L. F. Tribble, 1990, in a study which evaluated the effect of the organic copper in comparison with the copper sulphate. After absorption, copper is bond by albumin and ceruloplasmine. The concentration of the serum (plasma) copper reflects both the copper bound by the ceruloplasmine, and the “free” copper bound weaker by the albumin or by the small circulating peptides. The very high values noticed in the groups treated with Minitrex-Cu suggest that the copper chelated with amino acids had a beneficial effect on the gut flora and on forage digestibility. Because the minerals are bound by the chelation agents (such as amino acids), this chelate, Minitrex-Cu in our case, being more stable, interacts less in the digestive tract than the mineral ions. We may thus consider that Minitrex-Cu had a higher bioavailability than the copper sulphate and that it was stored in a higher amount in the organism.

Copper absorption may have also been influenced by other factors, such as the interactions with other antagonist substances, the dietary energy level, stress factors etc. Several scientific studies proved that the chelated minerals have a better bioavailability than the inorganic minerals. In a study on the efficiency of the copper bound to peptides and amino acids, used in pig feeding (Hill, K. E. Lloyd, T. A. Armstrong and T. E. Engle, 2004), also reported a higher absorption of the dietary copper. Given the synergic action of the copper and iron within the process of red cell (haemoglobin) formation, which easies Fe absorption, with no copper the iron cannot be included into the haemoglobin, so that we also monitored the evolution of blood iron concentration depending on the two dietary sources of copper.

The level of iron serum concentration increased with the dietary supply of copper, as seen in Figure 1.

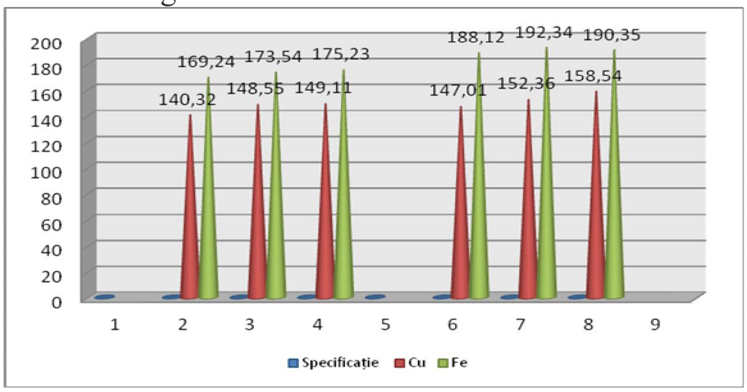

Figure 1- Serum concentration of copper and iron

This entitles us to say that transferrin doesn't play just the role of protein carrier for iron. In all groups treated with Minitrex-Cu, the values of iron concentration had significantly higher differences compared to the groups treated with copper sulphate (L1S, L2S, L3S), however, without exceeding the normal limits for this category of pigs. Taking into consideration that in piglets, the enzymatic equipment from the small intestine is poorly developed, and that the copper plays the role of activator of a large number of enzymes with role in absorption, bone development and body growth (Underwood and Suttle, 1999), the administration of a copper source with adequate bioavailability, to weaned piglets may have a positive influence on Fe absorption, thus improving the health state and maintaining the growth rate.The *average feed intake* increased slightly with the dietary copper level, without noticing significant differences between the two sources, as also reported by Coffey et al. 1994. *Body weight evolution and average daily weight gain dynamics* – no significant differences were noticed between the two sources, as also reported by Apgar et al. 1995 (Table 3).

Piglet performance (Table 3): body weight in the end of the experiment, the feed intake and weight gains showed no significant ($P \leq 0.05$) differences for the two sources of copper, contrary to the data reported by Coffey et al. 1995.The average daily weight gain was improved by the supplemental copper, the most efficient dose being 250 ppm, as also reported by Apgar et al. 1995. Although the improvement of the weight gain was determined partially by the increased ingestion, the dietary copper given in growth promoting dose also had a direct effect on feed conversion, irrespective of the copper source. The *frequency of enterocolitis*, as shown by the graphical interpretation of the data recorded in the daily health cards, indicates that the lowest incidence was recorded in the groups treated with the highest dose of copper, L3S and L3M, which allows us to say that copper has proven its antibiotic role even under the chelated form.

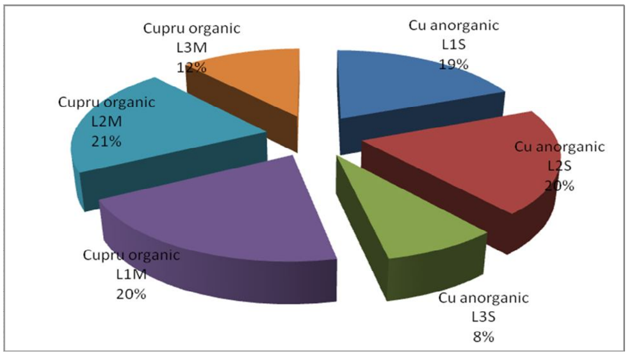

Figure 2- Frequency of enterocolitis

Table 3

Animal performance

Specification	Inorganic source			Organic source		
	L1S	L2S	L3S	L1M	L2M	L3M
Feed conversion ratio	1.52	1.47	1.41	1.51	1.47	1.42
Initial live weight(Kg)	8.27	8.25	8.23	8.27	8.36	8.27
Final live weight (Kg)	16.81[a]	17.16[a]	17.71[a]	16.74[a]	17.34[a]	17.97[a]
Average daily weight gain (kg/piglet/day)	0.388[a]	0.405[a]	0.431[a]	0.385[a]	0.408[a]	0.441[a]

Same superscript within the same row shows not significant differences ($P \geq 0.05$)

Copper concentration in the faeces (Figure 3): copper concentration in the excreta had the highest values in the groups treated with copper sulphate: L1S – 37.50 mg/Kg; L2S – 112.32 mg/Kg; L3S - 187.26 mg/Kg.

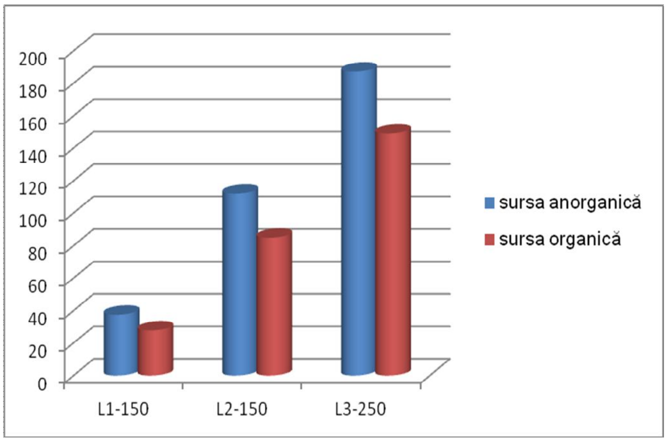

Figure 3- Copper excretion in the faeces

In all groups treated with Minitrex-Cu, the level of copper concentration in the faeces was lower than in the groups treated with copper sulphate, as follows: copper concentration was 8.63 mg/kg lower in L1M than in L1S, which received the same dietary amount of copper, but in inorganic form; copper concentration was 27.42 mg lower in in L2M than in L2S, while copper concentration was 37.87 mg lower in L3M than in L3S.

The groups treated with chelated Cu, the volume of excreted copper was 20-25%, in agreement with the findings of Veum, T. L. 2007 who used proteinated copper versus copper sulphate. These values suggest that the use of similar amounts of copper, or even lower than the tested amounts, but as chelates, piglet performance is not affected. Smits, R. J., and D. J. Henman, 2000, showed that the dietary minerals given to pigs can be reduced or replaced, provided they are added in organic form, when the costs are similar with the use of inorganic minerals; however, the environmental risk is much lesser.

CONCLUSIONS

The experiment showed that the use of copper chelates in the diets for weaned piglets decreased by 20-25% the amount of excreted copper without affecting piglet performance.

The blood profile improved in the groups treated with Minitrex-Cu, maintaining within the values specific for this species and category. Copper displayed its antibiotic power by the lower incidence of enterocolitis in the piglets immediately after weaning. Throughout the experiment there was a relationship of direct proportionality between the ingested and excreted amounts of copper.

These results are further evidence that nutrition plays a key role in the accomplishment of the policies aiming to minimalize the copper inputs into the soil in the vicinity of the pig farms. As Kornegay and Harper (1997) showed, a concept has to be developed by which diet formulation must be cost-effective and aiming to alleviate the adverse environmental impact on the soil and phreatic water.

We consider that diets L1M, L2M and L3M are in agreement with this concept because they provided the amount of copper which maintained piglet performance at the level obtained using the dietary copper sulphate, while decreasing the copper excretion by about 20-25%.

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