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Effects of Dietary Botanical Mix on Excretion and Tissue Levels of Some Trace Minerals (Cu, Fe, Mn, Zn) in Weaned Piglets

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Abstract

The decrease of some trace minerals (Cu, Fe, Mn, Zn) concentration in faeces from weaned piglets, by partially replacing the inorganic forms of trace minerals in the diet with a botanical mix: Jerusalem artichoke, (Helianthus tuberosus) and buckthorn (Hippophae rhamnoides), was studied. Two experiments were conducted: a mineral balance experiment performed on piglets housed in metabolic cages and a second one involving piglets housed under farm conditions. The piglets were assigned to 2 groups for each experiment (CB and EB for the balance experiment; CF and EF for the farm experiment), housed in individual metabolic cages, respectively in collective pens, fed on corn-soybean meal-based diets. The diet of the C groups included 1% inorganic mineral premix and contained 25 mg·kg^{-1}Cu. The diet of the E groups included 3% botanical mix and mineral premix having 50% less Cu, Fe, Zn, Mn salts. The experimental data show that, the 50% reduction of the minerals included in the premix for E groups, decreased significantly ($P \leq 0.05$) the trace elements concentration in excreta. The coefficients of apparent absorption of the minerals were comparable between CB and EB. At the end of experiments (d 26) the values of trace elements concentrations in the main organs of storage (liver, spleen) were comparable between groups.

Keywords: *Helianthus tuberosus, Hippophae rhamnoides, trace minerals, faeces, liver, spleen*

1. Introduction

Weaning is a complex stage in the life of pigs, which brings stress and which affects largely the feed intake, gastrointestinal tract development and the adaptation to solid food. The antibiotics and trace elements used for weaned piglets feeding helped them to go over the weaning crisis under efficient physiological and economic conditions [1]. Copper is one of the trace elements essential for growth, bone development and for reproduction [2]. It plays multiple systemic functions in animals, many of them associated to growth [3]. The Cu requirement for the weaned piglets is 5 - 6 mg·kg^{-1} feed [4], but the recommendations issued by nutritionists exceed this level [5]. For Cu, this overdose may reach hundreds or even thousands mg metal/kg, because this trace element is used as growth promoter instead of the antibiotics. Thus, important amounts of Cu but also of other trace elements appear in farm animal's excreta. Those minerals once accumulated in the soil have toxic effects on microorganisms and plants, becoming an environmental problem in the areas where animal rearing is intensive [6]. A reduction of Cu amount in piglets diets represents a way of reducing these environmental risks, while not hindering animal performances. Currently, the copper sources authorized for use in animal diets are inorganic salts of trace elements and Cu chelates of hydroxy analogue of methionine [7]. Anyway, in the absence of reliable analytical procedure to identify the form in which organically-bound minerals exist either in the diet or in the digestive tract, it is difficult to conclude on the efficiency of these products [8]. Natural feed additives of plant origin are generally believed to be safer, healthier and less subjected to hazards for humans and animals [9]. Mixtures of complex compounds, vitamins and minerals found in plants tend to work together synergistically. These combinations were more effective than when they were each used in isolated form. These beneficial effects make them useful as potential natural animal feed additives. Jerusalem artichoke (*Helianthus tuberosus*) is a phytoadditive and it was studied in animal feeds as replacer of antibiotic growth promoters. Its growth promoting effects are accomplished by suppressing pathogenic microbes and balancing gut microflora leading to a better nutrient absorption [10]. Sea buckthorn (*Hippophae rhamnoides*) is a true natural vitamin-mineral premix [11], and digestive function in weaned piglets was improved by using sea *Hippophae rhamnoides* extracts in diets [12]. The purpose of this study was to evaluate the effect of partially replacing the inorganic forms of trace minerals in the diet with a botanical mix (*Helianthus tuberosus* tubercles and *Hippophae rhamnoides* leaves*)*, on some trace minerals (Cu, Fe, Mn, Zn) excretion and deposition in weaned piglets. Two experiments were conducted, the first one being a mineral balance experiment performed on piglets housed in metabolic cages, while the second one involved piglets housed under farm conditions.

2. Material and method

Balance experiment (Exp B)

Animals, Housing and Treatments

The experiment was performed in compliance with Directive 2010/63/EU on the protection of animals used for scientific purposes and all procedures described, were approved by Ethical Commission of National Research and Development Institute for Biology and Animal Nutrition. The experiment used 8 Landrace × Large White half-brothers, male, castrated pigs with an average initial weight of 12.4 ± 0.3 kg. The experiment ran for 26 days. Throughout the experimental period, the piglets were randomly assigned to 2 groups, kept in individual metabolic cages (Agrico, Rybarska, Czech Republic) with an area of 0.87 m^2, placed in an experimental house under controlled environmental conditions (temperature of 24 ± 1^0C, humidity 50-60 %). The piglets were fed the respective diets daily, at 8.00 a.m. Water was supplied *ad libitum via* drinking nipples. The piglets were fed *ad libitum* on 2 experimental isoprotein and isocaloric diets (Table 1). The corn-soybean control diet (CB) was a conventional formula for the starter piglets, with 1% premix. Compared to CB diet, the experimental diet (EB) contained a botanical mix consisting of *Helianthus tuberosus* and *Hippophae rhamnoides*. Diet EB was supplemented with premix (1%), having 50% less Cu, Fe, Zn, Mn salts compared to the premix from diet CB. The vegetal materials used to produce this botanical mix are by-products from the production of natural food supplements. The botanical mix was used as powder and it was produced from vegetal material grown without any chemical stimulators. It was produced from the cakes resulting from the pressed *Helianthus tuberosus* tubercles which were mixed with *Hippophae rhamnoides* leaves. According to the standard procedures, at the age of 2 days, the piglets were injected with 200 mg Fe dextran® (Institute Pasteur, Bucharest, Romania). At the end of experiment (day 26) the piglets were slaughtered and the organ samples were collected. The pigs were weighed just before euthanasia.

Cu and other microelements level in botanical mix and experimental diets

The botanical mix was included in the compound feed at a level of 3% and the chemical composition is presented in table 1 and the compound feed structures are presented in table 2. The trace minerals (Cu, Fe, Mn, Zn) concentrations from the 2 experimental diets exceeded the requirements specific to the species and category and are detailed in table 3.

Performance

The daily records of feed intake were used to calculate the total feed consumption and the average daily intake. The piglets were weighed individually at the beginning, weekly and at the end of experiment to determine their weight and average daily gain.

Table 1. Chemical composition of the botanical mix

Nutrient	**Botanical mix**
Crude Protein (%)	9.58
Crude fat (%)	1.50
Crude fiber (%)	9.94
Inulin (%)	3.64
Calcium (%)	0.61
Phosphorus (%)	0.21
Copper (mg·kg^{-1})	**8.69**
Iron (mg·kg^{-1})	479.35
Manganese (mg·kg^{-1})	59.22
Zinc (mg·kg^{-1})	22.47

*Provided per kg of diet: vitamin A 10000 IU, vitamin D_3 2000 IU, vitamin E 30 mg, vitamin K_3 3mg, vitamin B_1 2 mg, vitamin B_2 6 mg, Ca pantothenate 13,5 mg, nicotinic acid 20 mg, vitamin B_6 3 mg, vitamin B_7 0,06 mg, vitamin B_9 0,8 mg, vitamin B_{12} 0,05 mg, vitamin C 10mg, Mn 30 mg, Fe 110 mg, Cu 25mg, Zn 100 mg, Co 0,3 mg, KI 0,38 mg, Se 0,36 mg.

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Table 2. Compound feed structure and quality indices

Ingredients	**C (%)**	**E (%)**
Corn	64.15	60.15
Sunflower meal	8.00	8.00
Soybean meal	14.00	14.00
Gluten	2.00	2.00
Milk powder	5.00	5.00
Oil	1.80	2.80
Monocalcium phosphate	1.40	1.40
Calcium	1.75	1.75
Salt	0.20	0.20
Methionine	0.10	0.10
Lysine	0.50	0.50
Choline	0.10	0.10
Premix *Zoofort* *	1.00	-
Premix *Zoofort (*with 50% Cu, Z, Fe, Mn reduced)**	-	1.00
Botanical mix	-	3.00
Analysed:		
Crude protein (%)	18.52	18.48
Metabolisable energy (MJ/ kg)	13.49	13.29
Calcium (%)	1.91	1.89
Phosphorus (%)	0.80	0.83

Table 3. Dietary Cu, Fe, Mn and Zn concentration

Trace element	**C** (mg·kg^{-1})	**E** (mg·kg^{-1})
Cu	**34.21**	**21.95**
Fe	199.89	158.59
Mn	45.69	31.83
Zn	134.69	84.91

NRC [4] requirement: 5 mg·kg^{-1} Cu; 80 mg·kg^{-1} Fe; 3 mg·kg^{-1} Mn; 80 mg·kg^{-1} Zn

Faecal, Urine, Leftovers and Tissue Collection for mineral balance

After a period of 5 days for accommodation, the mineral balance was determined weekly during 3 periods of 5 days each. The amount of feed given to each pig was weighed daily, as well as the leftovers (collected each morning). During the 3 periods of balance (5 days/ week) samples of ingesta and excreta (faeces and urine) were collected daily from each animal and average weekly samples were formed and assayed for Cu content. The faeces were collected once a day and stored at 3^0C. At the end of the collection period the faeces were weighed and homogenised; the feed samples were dried at 65^0C and ground. The urine volume was recorded daily and 10% of it was kept in containers at 3^0C. Sulphuric acid, 10 mL 10% was added to each container holding the urine. Average weekly samples of faeces/urine per piglet were formed at the end of each 3 periods of balance and stored at -18^0C.

The coefficients of apparent absorption, retention and utilization of Cu, were calculated using the data from the chemical analysis on the feeds, urine and faeces, corroborated with the daily records of the intake and excreta, using digestibility equations [13].

Samples of liver and spleen were collected from each slaughtered animal and stored at -80^0C until mineral analysis.

Experiment under farm conditions (Exp F)

Animals, Housing and Treatments

The experiment used 16 Landrace × Large White half-brothers, male, castrated pigs with an average initial weight of 11.84 ± 0.8 kg. The experiment ran for 26 days.

The piglets were fed on 2 experimental isoprotein and isocaloric diets as in the mineral balance experiment (Table 1). Throughout the experimental period the piglets were randomly assigned to 2 groups (control group – CF and experimental group – EF). The experiment was performed under farm conditions; the piglets from each group were housed in 2 collective pens (8 piglets/pen), with an area of 2 m^2/pen. The piglets were fed the respective diets daily, at 8.00 a.m., *ad libitum*, by using conventional open - front feeding area. Water was supplied *ad libitum via* drinking nipples.

Performance

All feed management and criteria used were described in the mineral balance experiment. At the end of the experiment (day 26) 4 piglets from each group were slaughtered and blood and organ samples were collected using the protocol described for the mineral balance experiment. Average weekly samples of faeces were collected and formed per group and prepared for the determination of Cu concentration. The faeces samples were weighed, homogenized and dried at 65^0C and ground.

Laboratory Analyses

The samples of diets, faeces and organ samples were analysed for mineral concentrations using a flame atomic absorption spectrometer Thermo Electron – SOLAAR M6 Dual Zeeman Comfort (Cambridge, UK) after the microwave digestion, as described by [14].

Statistics

The analytical data were compared performing analysis of variance (ANOVA), using STATVIEW for Windows (SAS, version 6.0). The differences between mean values in the groups were considered significant at $P<0.05$.

3. Results and discussion

Pig manure contributes to soil mineral concentrations and it can produce negative environmental impact. There is an increasing concern regarding Cu and Zn as they accumulate in the top layer of the soil. A major contributing factor to higher levels of Cu in the environment is the supplementation of pig diets with growth-promoting levels of these minerals [15]. Strategies to reduce the excretion of minerals by pigs and enhance the overall efficiency of pig production include the use of more bioavailable mineral sources. Some studies have shown a higher mineral retention of organic minerals [16, 17], than of inorganic forms. Several researches have focused on the possibilities of improving the mineral status of animals by using natural feed additives [18, 19, 9]. In the present study, the premix concentration of trace elements (Cu, Fe, Mn, Zn) was reduced by 50% and a dietary botanical mix of *Helianthus tuberosus* and *Hippophae rhamnoides* was added in order to obtain a significantly decrease of Cu concentration in faeces from weaned piglets. This botanical mix was chosen for its nutritional and medicinal value and because it contains various kinds of nutrients and bioactive compounds including vitamins, fatty acids, free amino acids and trace elements. The concentration of trace elements in the raw ingredients of the basal diet exceeds NRC [4] requirement for this category of piglets, as also reported in the literature [2]. However, the trace elements from the raw ingredients have a poor bioavailability mostly due to the presence of phytates [20]. This is why the diets are commonly supplemented with mineral salts. The dietary Cu concentration was provided through the raw materials from the basal diet (9.21 $mg \cdot kg^{-1}$ for C group and 8.82 $mg \cdot kg^{-1}$ for E group), through the botanical mix (0.26 $mg \cdot kg^{-1}$ for E) and through the copper sulphate (25 $mg \cdot kg^{-1}$ for C and 12.5 $mg \cdot kg^{-1}$ for E) from the mineral premix (Table 3). The 50% reduced concentrations of Cu, Fe, Mn, Zn in the premix lead to lower trace minerals concentrations in the E diet, by 35.8% for Cu, 20.7% for Fe, 30.3% for Mn and 36.9% for Zn, compared to C.

Effect of the dietary botanical mix supplement on the bioproductive parameters

The average daily gains (ADG) of the piglets from groups EB, CF, EF were significantly ($P<0.05$) lower than the gain from group CB (control group - piglets housed in digestibility pens). No significant differences were noticed between groups from the farm experiment regarding animal performance [19]. The lower supply of trace elements salts in the premix for the experimental groups decreased significantly ($P<0.05$) the average daily gains for groups CF, EB and EF compared to group CB, but their bioproductive parameters were within the normal limits for that category of animals [21]. A study on weaned piglets showed that the addition of fructooligosaccharides (chemical compound present in *Helianthus tuberosus*) into the diet of monogastric animals brings about several metabolic and physiologic changes, including improvements in feed efficiency, reduced diarrhoea, and reduced smell in faeces. These facts have been attributed to a change in the make-up of the intestinal microflora population [22].

Effect of the dietary botanical mix supplement on copper balance (balance experiment)

Copper balance was conducted on piglets housed in individual metabolic cages. This allowed the daily quantification of the ingested feeds and the amount/volume of eliminated faeces/urine. Both the amount of absorbed Cu (mg/day) and the amounts of retained Cu (mg/day) were significantly ($P\leq0.05$) lower in group EB compared to groups CB (Table 4). However, the absorption and retention coefficients didn’t differ significantly ($P>0.05$).

No significant differences ($P>0.05$) between the groups in terms of digestibility coefficients show that the diet formulation given to group EB is a potential feeding solution to reduce the amount of Cu from piglet excreta, which is a very good thing, given the fact that this heavy metal is a long-term pollutant of the environment [23]. A possible explanation, could be the presence of inulin-type fructans concentrations in *Helianthus tuberosus*, considered to be prebiotics, which are carbohydrates that are not digested by vertebrates and that interact selectively with fermentation in the intestine. It was shown that there is an increase in the intestinal absorption of minerals due to indigestible carbohydrates [24, 25]. The differences between the amount of Cu absorbed by group CB compared to EB are due to the higher dietary Cu level in this group (25 ppm Cu for CB compared to 12.5 ppm Cu for

EB). The same explanation goes for the differences between the two groups in terms of retained Cu. No significant differences (P>0.05) were noticed between groups regarding apparent digestibility coefficients of Fe, Mn and Zn (data not shown). The ingested, excreted, absorbed and retained amounts of Fe, Mn and Zn were significantly increased (P≤0.05) for CB group compared to EB. These results sustain observations presented before (table 4) and they are expected due to higher dietary minerals level in the diet of CB group.

Table 4. Cu balance data (average values/group)*

	CB	**EB**	**SE**	**P value**
Botanical mix (%)	**0**	**3**		
Cu (CuSO4) added via the premix (mg·kg^{-1})	**25**	**12.5**		
Copper				
Intake (mg/day)	56.57	40.79	3.90	0.0328 S
Faeces (mg/day)	34.55	25.95	5.07	0.0475 S
Urine (mg/day)	0.46	0.29	0.07	0.0471 S
Absorbed (mg/day)	22.02	14.84	1.84	0.0427 S
Retained (mg/day)	21.57	14.54	1.80	0.0430 S
Absorption (%)	38.68	36.37	1.62	0.5090
Retention (%)	37.91	35.65	1.68	0.5353
Utilization (%)	97.88	97.81	0.37	0.9385

*Mean values within a row were significantly different (P<0·05).

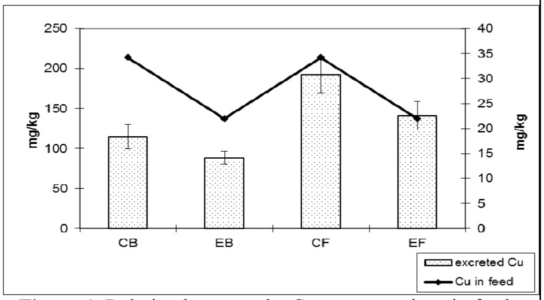

Figure 1. Relation between the Cu concentrations in feed and Cu excreted

Effect of the dietary botanical mix supplement on the copper concentrations in excreta

The amounts of Cu eliminated through the faeces and urine differed significantly between the groups (Fig. 1). In the groups with botanical mix supplements and dietary Cu, Fe, Mn, Zn concentration reduced by 50% (EB and EF), as expected, the amount of Cu excreted through the faeces and urine was significantly lower compared with the respective C groups (Fig. 1). In the experimental diets (EB and EF), the Cu concentration from the premix of E groups was reduced by 50% compared to C groups. Within this context, the Cu concentration in the faeces collected from group EB was 22.62% lower compared to group CB, while for group EF, the concentration was 26.65% lower compared to group CF. The Cu level from the excreta of piglets assigned to group EF (farm experiment) was 64.2% higher compared to group EB (balance experiment), which suggests a lower absorption of the Cu by the farm piglets. For the other three trace elements studied (Fe, Mn, Zn) the minerals excretion was also significantly lower for EF compared to CF [19] and significantly higher for EF compared to EB (32.13% for Fe; 57.21% for Mn; 46.28% for Zn). These observations, correlated with the data on piglets' performance, are supported by the hypothesis of a minimal influence of the pathogens in the case of the balance experiment. Other authors showed that, under certain conditions, inulin-type fructants can enhance mineral absorption by their impact on improving intestinal health or by stabilizing intestinal flora. The effects of inulin supplements extracted from chicory root on the absorption of trace elements [26] and on the immune response in weaned piglets [27] were also investigated. NOVACK, 2007 [28] has pointed out that by improving the intestinal health, prebiotics increase the absorption of nutrients. The mineral concentrations found in the faeces samples from farm experiment (CF and EF) were significant higher than recorded for balance experiment (CB and EB).

Effect of the dietary botanical mix supplement on Cu concentrations in organs

At the end of experiment (d 26) the values of Cu concentration in the main organs of storage (liver and spleen) were comparable between the groups from the same experiment. Significant differences ($P \leq 0.05$) were noticed regarding iron and zinc concentrations in liver and iron concentrations in spleen, between groups from different experiments (Table 5). The matrix presented in Table 6 shows a very good correlation between the bioproductive parameters of Cu status. The matrix was built using the experimental data from both types of experiments.

Table 5. Trace elements concentration in organs (average values/group)

Botanical mix (%)	**CB**	**EB**	**CF**	**EF**	**SE**	**P value**
Cu ($CuSO_4$) added via premix (mg·kg^{-1})	**025**	**312.5**	**025**	**312.5**		
Liver						
Copper	20.47	19.42	18.90	17.60		0.0589
Iron	254.84	246.44	158.97	153.63	0.432	<0.0001S
Manganese	12.02	12.95	11.50	11.96		0.0693
Zinc	168.73	164.91	182.88	186.51	12.66	0.0367S
Spleen					0.209	
					.395	
Copper	3.32	3.23	3.19	3.09		0.3094
Iron	339.07	323.49	234.97	232.56	0.055	<0.0001S
Manganese	1.78	1.73	1.69	1.62		0.4815
Zinc	100.64	97.66	97.42	93.60	14.95	0.8732
					0.068	
					10.82	

Table 6. Correlation matrix between the data from the 2 experiments

	Intake	**Gain**	**Cu in liver**	**Cu in spleen**
Intake	1.000	0.876	0.986	0.983
Gain	0.876	1.000	0.930	0.941
Cu in liver	0.986	0.930	1.000	1.000
Cu in spleen	0.983	0.941	1.000	1.000

The increased mineral excreta and the decreased mineral deposition are the consequences of a lower absorption of the trace elements in the farm experiment compared to the balance experiment. Because the dietary formulations were similar for the two experiments these differences may be attributed to the different experimental conditions. HYUN & al. [29] showed that the size of the pen is one of the stress factors which affect piglet performance. The piglets from experiment 1 were housed in individual crates with an available area of 0.87 m^2/animal, individual feeding, *ad libitum*. The piglets from experiment 2, managed according to the usual rearing technology, were kept in pens (8 piglets per pen), with an available area of 0.25 m^2/animal, with open-front feeding. Another explanation could be that in the case of the balance experiment, the hygiene conditions are more tightly controlled so that the possibility for the development of pathogens is lower than when the animals are reared under farm conditions.

4. Conclusions

A 50% reduction of copper sulphate ($CuSO_4$) in the premix for the experimental groups EB and EF, compared to the usual amount (25 mg·kg^{-1} diet) used for the piglets from groups C, decreased significantly the concentration of Cu in the excreta of these piglets. Overall, the piglets reared under farm conditions (CF and EF) performed less well than the piglets from the balance trial but, nevertheless, these values were within the normal range for this category of piglets. Further research is needed in order to give a resolute conclusion on the efficiency of the dietary botanical mix of *Helianthus tuberosus* and *Hippophae rhamnoides*, this study being just the first step. These researches are needed because they allow the use of by-products from the production of natural plant supplements for human consumption, in piglet feeding, while generating solutions for environmental protection.

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