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Research Article

The Importance of Bioactive Feed Additives in Feeding Pigs and Their Impact on the Digestibility of Particular Nutrients [1]

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Abstract

In recent years, bioactive additives have become a subject of interest in the field of nutrition of farm animals. Therefore, the application of biosorptive properties of components is an alternative to inorganic mineral compounds and can play an important role in innovative nutritional strategies. The study presents a quantitative measurement of mineral components such as manganese, zinc, copper, and iron by using the scanning electron microscopy with an energy-dispersive X-ray spectroscopy (SEM-EDX). The research material was the feces collected from 39 growing-finishing pigs divided into 3 groups: control one, receiving inorganic mineral compounds, and experimental groups, fed organic mineral mixtures constituting 100% and 125% of the recommended daily intake. Each group of animals consisted of 13 animals (n=13). The results showed a difference in the elemental composition of the pooled samples of all three groups. The highest bioavailability of minerals had the group receiving 100% of the recommended dose of the experimental organic compounds. In the control group, the differences were statistically significant ($P<0.05$) in the case of manganese, zinc and iron. The study showed that the mineral supplements obtained in the biosorption process showed a high rate of absorption from the gastrointestinal tract and did not require the intake of doses greater than 100% of the daily requirement. The high proportion of trace elements in the second experimental group did not increase their bioavailability and contributed to the reduced digestibility of nutrients.

Keywords: *Biosorption, Trace minerals, Pig, Digestibility*

Domuz Beslenmesinde Biyoaktif Yem Katkı Maddelerinin Önemi ve Özellikle Besinlerin Sindirimi Üzerine Etkisi

Özet

Biyoaktif katkı maddeleri son yıllarda çiftlik hayvanlarının beslenme alanında önemli bir konu haline gelmiştir. Bu nedenle bioabsorptif maddeler bileşenlerinden dolayı uygulamada kullanılan inorganik mineraller için alternatif oluşturmakta ve yenilikçi besleme stratejilerinde önemli bir rol oynamaktadır. Bu çalışmada enerji dispersif X-ışın spektroskopisi (SEM-EDX) ile desteklenmiş, taramalı elektron mikroskobu kullanarak manganez, çinko, bakır ve demir gibi mineral bileşenlerin nicel bir ölçümü sunulmuştur. Araştırmada kullanılan dışkı, 39 adet ve 3 gruba ayrılmış deney hayvanlarından toplanmıştır. Kontrol 1 grubu inorganic mineral bileşenleri aldı ve 2 deney grubu, %100 ve %125 inorganik mineral içeren bileşiklerden oluşturulan yemle günlük beslendi. Her bir grup 13 hayvandan (n=13) oluşmuştur. Sonuçlar oluşturulan havuzlarda toplandı ve her üç grupta da farklılık gösterdi. Bioabsorptiflik; minerallerin önerilen dozunun %100 olduğu grupta en yüksek seviyede oldu. Kontrol grubunda, farklılıklar; manganez, çinko ve demir durumunda istatistiksel olarak anlamlı ($P<0.05$) bulundu. Çalışma, mineral takviyelerinin büyük dozlarda uygulandığında gastrointestinal sistemde bioabsorptifliğinin düşmesi nedeniyle %100 ün üzerinde alınmasının gerekli olmadığını gösterdi. İkinci deney grubunda eser elementlerin yüksek porsiyonda alımı bioabsorptifliği yükseltmemiş ve besin sindirilebilirliğini azaltmıştır.

Anahtar sözcükler: *Bioabsorpsiyon, İz mineraller, Domuz, Sindirilebilirlik*

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INTRODUCTION

Current intensive pig production is focused on maximum slaughter efficiency of these animals [1,2]. Optimal nutrition plays a decisive role in breeding, as it ensures proper growth and development of the young organism. Concentrated feeds used in the feeding of livestock, such as corn, oats or soybean meal do not fully cover the demand for essential minerals and vitamins. On the other hand, inorganic mineral supplements often contain a higher concentration of these compounds with respect to the recommended daily requirements [2-4]. Production of increased volume of manure and limited utilization area can cause environmental pollution. Nutritional strategies formulated as concepts of cost-effective diets include meeting the mineral demand, increased efficiency of feed used, and high bioavailability of minerals with their minimal excretion [5]. Nutritional deficiencies resulting from the presence of the low absorbable minerals (i.e., oxides, chlorides, sulfates, nitrates) are the cause of increased mortality of the litters, dermatological diseases, fertility disorders, increased susceptibility to infections, or diseases of the musculoskeletal system such as rickets and abnormal ossification [6-9]. In recent years, special attention is paid to the bioavailability of minerals, and thus the degree of absorbability from the gastrointestinal tract [10]. Absorption of individual micro- and macro-elements depends, among others, on their origin [6,9,11]. Since the bioavailability of inorganic salts is relatively low, there is a need to seek alternative sources of minerals, which ensure their high bioavailability [6,12]. Production of high quality chelates is difficult and expensive, which is reflected in the high price of the products based on chelate compounds. In addition, there have been reports of an irritating effect of chelates on the gastrointestinal tract of animals [12]. Utilization of the biosorprtive properties of the substrates is an alternative both for chelates and inorganic substances [13]. The technology of biosorption process is based on binding metal cations by functional groups on the surface of the biomass and the formation of a complex in the donor-acceptor system [14-16]. Nutritional analyses carried out on laying hens and growing-finishing pigs, with the use of algae biomass enriched with selected micronutrients, confirmed the beneficial health effects and increased bioavailability of trace elements applied [17]. Since the main feed in the feeding of growing-finishing pigs is soybean meal, constituting a source of valuable protein, the use of its biosorpitve properties can simultaneously contribute to an increase of the nutritional value of the mixture [7]. Increasing the degree of absorption of mineral substances from the digestive tract is reflected in digestion studies conducted to determine the amount of excreted nutrients in the feces [9]. There is also a possibility of relatively rapid implementation of a quantitative measurement of the elements in the material studied using scanning electron microscopy with an energy-dispersive X-ray spectroscopy (SEM-EDX). SEM-EDX method is now widely recognized as a tool, which is applied in many scientific disciplines [18-23]. This technique allows precise identification and imaging of the distribution of ions of chemical elements being a part of the organic material under test. Another advantage is the high sensitivity of these measurements, the possibility of repetition and data archiving. Moreover, SEM-EDX analysis, due to minimal interference with the physical and chemical properties of the test material, is referred to as a quasi-non-destructive method [18,20].

Given the current benefits of the biosorption process in obtaining bioactive additives, it was decided to enrich the post-extraction soybean meal in trace elements playing an important role in feeding the pigs. The aim of this study was to determine the influence of mineral additives on the digestibility of selected nutrients during the second period of fattening.

MATERIAL and METHODS

Biosorption Process

Soybean meal (Vetos Plant, Zębowice, Poland) was separately enriched with the following microelements: zinc (II), manganese (II), copper (II) and iron (II) via biosorption. The solutions were prepared by dissolving appropriate amounts of inorganic salts in deionized water ($ZnSO_4 \cdot 7H_2O$, $MnSO_4 \cdot H_2O$, $CuSO_4 \cdot 5H_2O$, $FeCl_2 \cdot 4H_2O$). The enrichment process was performed in a 0.1 dm^3 bed column reactor containing demineralized water, adjusted with NaOH/HCl (POCh, Gliwice, Poland) to pH 5.0, measured with pH meter equipped with an electrode (InLab413) with temperature compensation (Mettler-Toledo Seven Multi; Greifensee, Switzerland). Biosorption process was carried out at 20°C until complete saturation of the bed, controlling the concentration of the solution coming out from the column. Biomass after the process of enrichment was dried on air at 25°C for 48 h.

Feeding Experiment and Sample Collection

The feeding experiment was carried out on 39 pigs (line 990, females, 20-23 kg initial body-weight, 10-week-old) kept in individual cages in a room with controlled heating with the mean temperature 19±0.3°C and ventilation with the mean air speed 0.2±0.02 m/s. Pigs were weighed individually at the beginning and the end of the experiment. All individuals were given anthelmintic preparation and a vaccine immunizing against Porcine circovirus type 2 (PCV2). Feed and water were available semi *ad libitum* using automatic feeding systems-automatic bell drinkers and tubular feeders.

Pigs were randomly divided into three groups: two experimental and one control group. Both control and experimental groups were fed the same basic feed composition, differing only in feed premixes *(Table 1)*.

Table 1. *Composition and feeding value of mixtures*
Tablo 1. *Karışımların kompozisyonu ve besin değerleri*

Component	GROWER Content (g/kg)	FINISHER Content (g/kg)
Ground Triticale	300.0	300.0
Ground Barley	160.0	200.0
Ground Corn	200.0	200.0
Soybean Meal 46%	140.0	90.0
Rapeseed Meal	80.0	80.0
Wheat Bran	65.1	87.5
Soy Oil	20.0	9.0
Fodder Chalk	10.0	10.0
Premix	10.0	10.0
Monocalcium Phosphate	6.0	4.5
NaCl	3.5	0.3
Pell-Tech	3.0	0.3
L-Lyzine-HCl 99%	2.0	2.3
Xynalase 4000G	0.3	0.3
Phyzme XT	0.1	0.1
Chemical Composition		
Total Protein	170.0	154.0
Crude Fiber	43.0	45.0
Lysine	9.5	8.6
Methionine + Cysteine	6.0	5.6
Threonine	6.2	5.6
Tryptophan	2.1	1.9
Total Calcium	6.6	5.8
Total Phosphorus	6.2	6.0
Total Sodium	1.7	1.5
Metabolic Energy	129.0	126.0

** The composition of standard feed was established by the producer. The content of ingredients provided per kg of diet: vitamin A (retinyl acetate) 700.000 IU; vitamin D_3 (cholecalciferol) 50.000 IU; vitamin E (DL-a-tocopheryl acetate) 7.000 IU. IU- International Unit. Premix provided: Cu, 20 (as $CuSO_4·5H_2O$); Fe, 50 (as $FeSO_4·H2O$); Mn, 20 (as MnO_2); Zn, 50 (as ZnO) mg/kg of diet*

According to different nutritional requirements for growth of the animals, two different feed compositions were used, i.e., growers during the first period of fattening (40-65 kg) were fed a standard grower feed mixture, while in the second period of fattening (65-105 kg) they were fed a standard finisher feed mixture. The composition and feeding value of mixtures is presented in *Table 1*. The source of vitamins and micro elements was a commercially available premix produced by Cargill Poland ltd. (Kiszkowo, Poland).

The control group was fed a basal diet with micro-elements in inorganic form, while experimental groups were fed a diet supplement, in which microelements in inorganic form were eliminated at the production stage and substituted for enriched soybean meal. Soybean meal was enriched with Mn, Zn, Cu and Fe microelements. The portions of microelements were established according to European Union pig nutrition standards. The demand for microelements in the control group was covered in 100%, and in experimental groups in 100% and 125%. The experiment was carried out for 13 weeks.

Feces of all animals were collected during 5 days. The samples were stored in a refrigerator at 3-4°C for 5 days from the first collecting. Next, each separate group of samples was pooled together and prepared collectively, and then chemical analyses were performed.

SEM/EDX Analysis

The feces were collected from each pig, transported to the laboratory and analyzed. All samples were kept in the freezer until analysis. The content of the microelements in each sample was analyzed by scanning electron micro-scopy (SEM, Zeiss Evo LS 15) combined with energy dispersive X-ray analysis (EDX). The samples were analyzed in four replicates.

Prior to the SEM/EDX analysis, samples were washed with distilled water and dehydrated in a graded series of ethanol dilutions (POCh, Gliwice, Poland), from 50% to 100%, with a 10% gradation. Dried samples were subsequently placed in the microscope chamber. The quantax detector (Brüker) with 10 kV filament tension was used for SEM/EDX analysis. The values obtained were presented as weight percentage (wt %).

Statistical Analysis

Normality of data population was determined using Shapiro-Wilk test, whereas equality of variances was assessed using Levene's. Differences between investigated groups were analyzed using one- way analysis of variance (ANOVA). The arithmetic mean values, standard deviations and statistical analysis were performed using the Statistica 7.0 software (StatSoft, Inc., Statistica for Windows, Tulsa, OK). The values of $P \leq 0.05$ were considered significant.

RESULTS

SEM-EDX analysis allowed evaluating the elemental composition and average concentrations in all samples collected. The averages of weight percentage content were presented in *Fig. 1*, *Table 2* and *Table 3*.

Reduced weight percentage of all microelements was observed in both experimental groups in comparison to control. Only the content of Zn (29% in comparison to control) and Cu (15% in comparison to control) were higher in samples from the group where the demand for microelements was covered in 125%. Lower concentration of microelements in experimental groups, when compared to control, was statistically significant ($P \leq 0.05$) in the case of Mn (P=0,031), Fe (P=0.0048) in Group I, and Mn (P=0.0003) in Group II.

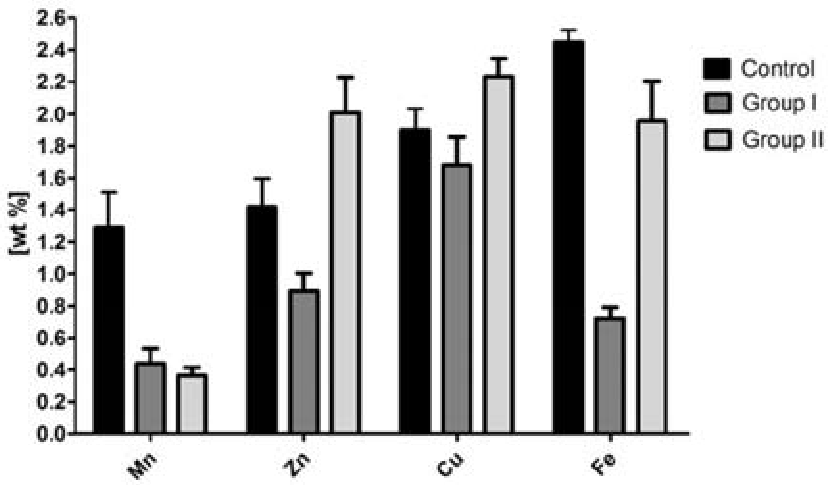

Fig 1. The averages of weight percentage content of microelements in control and experimental groups (Group I and II). The statistically significant differences were found in the experimental groups in comparison to controls

Şekil 1. Mikroelementlerin kontrol ve deneysel gruplarda (Grup I ve Grup II) ağırlıktaki oranı. Kontrol grubu deneysel grupla karşılaştırıldığında istatistiki olarak önemli farklar bulunmuştur

Table 2. *Comparison of the effects of soybean meal additive covering 100% (Group I) and 125% (Group II) requirement for the content of minerals in the sample*

Tablo 2. *Soya unu katkı maddesinin etkilerinin karşılaştırılması; % 100 (Grup I) ve % 125 (Grup II) numunelerde gereksinim için mineral içeriği*

Group	Mineral	Differences in Percentage [%]	P Value
Control and Group I	Mn	66	0.0031 significant
	Zn	37	0.10 not significant
	Cu	12	0.15 not significant
	Fe	71	0.0048 significant
Control and Group II	Mn	72	0.0003 significant
	Zn	42	0.30 not significant
	Cu	18	0.005 significant
	Fe	20	0.13 not significant
Group I and Group II	Mn	21	0.23 not significant
	Zn	56	0.007 significant
	Cu	25	0.0058 significant
	Fe	63	0.004 significant

Table 3. *The average content of microelements in swine feces in the control and experimental groups*

Tablo 3. *Deney ve kontrol gruplarında domuz dışkısı mikroelementlerinin ortalama içeriği*

Group	Mn		Zn		Cu		Fe	
	Mean wt%	SD	Mean wt%	SD	Mean wt%	SD	Mean wt%	SD
Control	1.28	0.220	1.41	0.18	1.9	0.13	2.44	0.07
Group I	0.44	0.091	0.89	0.11	1.67	0.18	0.72	0.07
Group II	0.36	0.005	2.00	0.421	2.23	0.015	1.95	0.68

The addition of microelements bound by biosorption caused a significant decrease in the content of Manganese in the pig feces; concentration of Mn was significantly higher in the control group in comparison to experimental groups. Similar results were obtained in the measurements of the content of Fe element. While the differences in the concentration of Mn between experimental groups were unnoticeable and not statistically significant, the difference in the content of Fe in group I and II was considerable. Interestingly, an increase in the content of Zn in group II was observed in comparison to control, and at the same time a decrease of wt % of zinc in group I.

The differences in the content of Zn, Cu and Fe between both experimental groups had $P \leq 0.05$ (Zn- $P=0.007$; Cu- $P=0.0058$; Fe- $P=0.004$) *(Table 3)*. A lower content of these microelements was clearly visible in group I. The differences between groups were readily noticeable. There was a decreasing tendency observed in the content of Zn, Cu and Fe in the samples from group I. The concentration of the content of Zn was 56% lower in group I compared to group II. Similar situation was observed for the concentration of Cu and Fe, as their content in group I was 25% and 63% lower, respectively. Interestingly, the higher content of Mn (21% higher than in group II) was observed in the group with microelement demand covered in 100%.

DISCUSSION

Modern manufacturing technology of organic mineral compounds based on the process of biosorption has been the subject of intense research. The main advantage of the resulting biocomplexes is a significant restriction of the competition between microelements for the same site of absorption. However, interactions between trace elements, based on synergistic or antagonistic action, have not yet been fully understood and require further studies [9]. Organic forms of trace elements are readily available for the body and contribute to the growth of their average content in the material tested [24-26]. The results of the chemical analysis in relation to the control group showed an increase in the average content of copper and zinc in the experimental group receiving 125% of the recommended daily intake of minerals. This indicated a reduced absorption and assimilation of these elements from the gastrointestinal tract. Some authors noted the strong antagonism between manganese and copper [9]. The present results indicated the highest average digestibility of manganese in the groups supplemented the organic additive of this element, which can directly affect the reduced absorption of copper from the gastrointestinal tract. What is more, there is also a strong competition between zinc and copper due to similar physical and chemical properties of these elements [27]. Effect of zinc on copper metabolism is manifested by a reduced availability of copper, and thereby increased excretion of this element in feces [9,27]. Our comparative analysis of the data obtained confirmed this concept.

An important role in the control of the absorption of iron and zinc in humans is played by manganese [28]. In all growing-finishing pigs studied, the highest absorption of this element was observed in both experimental groups. The average content of zinc in the group I was approximately 56% lower compared to group II, and the differences were statistically significant. Similarly reduced values were found in the group receiving 100% of the recommended dose for copper and iron. We postulate that increasing the amount of minerals by ¼ with respect to a daily requirement, does not increase the degree of absorption and assimilation from the gastrointestinal tract. Therefore, the addition of a higher concentration of minerals in the diet may increase the competitiveness of the elements, thereby impairing their homeostasis. The results obtained confirmed better utilization of the elements in the experimental group, in which the demand was covered in 100%. Our studies also confirmed the low level of utilization of minerals in the inorganic form in the test growing-finishing pigs.

In conclusion, these data indicate a higher bioavailability of minerals obtained in the process of post-extraction biosorption of soybean meal with regard to inorganic compounds. Bioactive mineral mixtures enriched with microelement cations can complement not only the deficiencies of these elements in the diet of livestock, but also contribute to an increase in the content of trace elements in animal products. Moreover, developing pig production systems are reaching global scale and become increasingly intensified. We postulate that the diets based on bioactive minerals can increase the safety of the environment by reducing pollution and their impact on the economy of production.

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