DEVELOPMENT OF A LOW-ZINC PIG MODEL FOR IMMUNOLOGICAL INVESTIGATIONS

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Summary. Zinc is an important component of the immune response and the immunoregulation of parasitic infection. In order to determine whether zinc deficiency can be achieved in pigs by feeding a diet high in phytic acid, thereby producing a model for studying the immune response to parasites, a feeding trial was conducted. The experiment was designed to investigate the effect of phytic acid on elemental zinc and alkaline phosphatase (AP) levels in the blood plasma and livers of growing pigs. Two groups of 4 pigs each were fed for 5 weeks on either a normal diet or the same diet supplemented with extrinsic phytic acid. The amount of zinc and alkaline phosphatase in blood plasma began to decrease markedly 3 weeks after pigs had keen fed phytic acid. After 5 weeks, pigs fed the phytic acid ration had blood zinc levels by 35% lower than the control pigs and 44% less AP. In addition, the levels of liver zinc decreased 22% after 5 weeks on the phytic diet. It was demonstrated that the feeding of phytic acid significantly reduced dietary zinc availability and that it can be employed experimentally to zinc-deficient growing pigs for different experimental purposes, and for studying zinc role in the immune response to parasitism.
Keywords: phytic acid, zinc deficiency, growing pigs.

FITINO RŪGŠTIES ĮTAKA CINKO TRŪKUMUI KIAULIŲ ORGANIZME

Santrauka. Cinkas yra svarbus mikroelementas, dalyvaujantis medžiagų apykaitoje ir reguliuojantis įvairiems patogenams imuninį atsaką. Eksperimentas atliktas siekiant sukelti cinko trūkumą kiaulių organizme, kai ateityje reikės atlikti parazitologinius ir imunologinius tyrimus. Tirta fitino rūgšties, sušertos kartu su pašaru, įtaka cinko trūkumui kiaulių organizme, nustatytas cinko ir šarminės fosfatazės kiekis kiaulių kraujo plazmoje ir kepenyse. Eksperimentas atliktas su aštuoniomis 2-4 mėn. amžiaus kiaulėmis iš SPF fermos. Tirtos kiaulės atsitiktinai suskirstytos į eksperimentinę ir kontrolinę grupes po 4 kiekvienoje. Kontrolinė grupė šerta 20% sojų pupelių, 40% avižų grūdų, 40% kviečių ir mineralinių medžiagų bei vitaminų priedu (pagal Danijos rekomendacijas augančioms kiaulėms); eksperimentinė grupė tuo pačiu pašaru su fitino rūgšties priedu (12g/kg pašaro). Nustatyta, kad cinko ir šarminės fosfatazės kiekis eksperimentinių kiaulių kraujo plazmoje palyginti su kontroline grupe ženkliai sumažėjo po 3 savaičių nuo bandymo pradžios. Be to, šeriant kiaules dieta su fitino rūgštimi 5-ąją bandymo savaitę, cinko kiekis kraujo plazmoje sumažėjo 35%, šarminės fosfatazės 44%, o kepenyse 22% palyginti su kontrole. Remiantis šio bandymo rezultatais galima daryti išvadą, kad fitino rūgšties priedas ženkliai sumažina cinko įsisavinimą gyvulio organizme sukeldamas ženklų cinko trūkumą kiaulių kraujo plazmoje ir kepenyse.
Raktažodžiai: fitino rūgštis, cinko trūkumas, kiaulės

Introduction. Zinc is a trace element essential for animals, plants and microorganisms (Baker, Ammerman, 1995). Animals obtain zinc from the diet such as cereal grains, other seeds, forages or from extrinsically supplied inorganic sources (Underwood, Suttle, 1999). Zinc plays a central role in the immune system, and it is well documented that zinc-deficient animals and humans have increased susceptibility to a variety of pathogens, including parasitic nematodes in intestinal and systemic sites (Scott, Koski, 2000; Shankar, Prasad, 1998). Zinc affects multiple aspects of the immune system, from the skin barrier to gene regulation of T and B lymphocyte function; zinc is crucial for development and function of cell mediated immunity, including neutrophil and macrophage activity (Scott, Koski, 2000). In addition to altering the immune status, zinc deficiency causes severe inappetence, growth depression and impaired reproductive performance in different animal species including pigs (Friis et al., 1997; Underwood, Suttle, 1999). Our research focuses on the importance of micronutrients on host resistance to parasites. Consequently, the objective of the experiment reported here was to establish not severely retarted zinc-deficient pig model for subsequent investigations on the immune response of the pig to parasites. The design chosen to achieve zinc depletion involved the feeding of a phytic acid-rich diet. Phytate or phytic acid is a naturally occurring, intrinsic phosphorus compound in all grains. Because phytate is not degraded by intestinal enzymes in monogastrics, it forms unabsorbable complexes with zinc and presumably calcium in the gut (Wedekind et al., 1994). The zinc dietary requirements of pigs are often expressed in relation to dietary phytate and calcium in the diet rather than to zinc concentrations *per se* (Baker, Ammerman, 1995) Under farm conditions animals may suffer from zinc deficiency if fed phytate-rich feeds and

calcium supplements (House, Welch, 1989; Tucker, Salmon, 1955). Elemental zinc in blood plasma is widely used as an indicator of zinc status (due to the lack of more specific indicators). Alkaline phosphatase is a Zn-dependent enzyme activity of which is susceptible to zinc status (Larsen, Sandström, 1993). Elemental zinc in liver tissue is an indicator of internal zinc deposition.

In this study we compared the influence of normal and phytate-enriched diets on blood and liver zinc levels with the aim of developing a zinc-deficient model for infection-immunity studies.

Material and Methods. The experiment included 8 specific-pathogen-free hogs (Landrace/Yorkshire Danish crosses). The pigs were divided into 2 equal groups (Groups 1 and 2). The two experimental diets used in the experiment are described in Table 1. The normal diet was based on Soya bean meal, barley and wheat with addition of minerals and vitamin mixtures according to standard recommendations; the phytate enriched diet was identical except that it was supplemented with 12 g of phytic acid (3Sigma, P 8810, Na-salt) per kg of diet. Group 1 pigs were fed the normal diet, and Group 2 pigs received the phytate-enriched diet. Both groups were fed these diets for 5 weeks. The pigs were weighed at the start of the experiment and further every week; the pigs were always weighed at the same time of the day (compared to feeding time). Blood samples were collected from the jugular vein for analyses. Sampling started the day after arrival and hereafter once a week until week 5.

Table 1. **The experimental diets, per kg diet.**

Feed	Group 1	Group 2
Soya bean meal, toasted, g	200	200
Barley, g	400	200
Wheat, g	400	400
Supplement of phytic acid, g		12

Minerals except zinc were supplied to fulfill Danish recommendations, i.e. Na_2HPO_4, $2H_2O$, 10,3 g; $CaCO_3$, 17 g; $Fe(II)SO_4,7H_2O$, 200 mg; $CuSO_4$, $5H_2O$, 20 mg; $Mn(II)SO_4,7H_2O$, 200mg; KI, 0.25 mg; NaCl, 3g. 10 g of maize oil was added per kg. Vitamins were supplied according to Danish recommendations for growing pigs. The vitamin formulations were kindly delivered by Roche Vitamins, Denmark.

Plasma zinc was determined by atomic absorption spectrophotometry (Perkin Elmer 5000) and plasma alkaline phosphatase (AP) was measured on an automated analyser (Advia 1650, Bayer) using the test kit Enzymatic/standardized DGKC (DEA) Rate reaction (Bayer). Both methods are subjected to daily internal quality control and external quality control (4 times per year).

At the end of the experiment (5 weeks after start), all pigs were slaughtered; the livers were quickly removed, separated from mesenteries, and frozen until further analysis. Representative samples, excised from different lobes of the organ were selected and dry ashed at 550 ^oC in a muffle oven. Ashes were dissolved in HNO_3/HCl (Suprapur/Analytical grade, 1: 3) according to, the method described by Larsen & Sandström (Larsen, Sandström, 1992). The analysis of Zn content in livers was performed using Automatic Analysis System.

Ethical consideration. The experiment was approved by the Danish Animal Ethical Committee (Experimental animal permission license: 2000/561-321). Meetings were held with the agricultural and laboratory technicians to explain the purpose of the experiment and the requirements for the persons handling the pigs.

Results. No overt clinical signs were observed in pigs throughout the experimental period. All pigs exhibited a normal appetite and daily feed allowances were completely consumed in both diet groups. Average live weight (standard deviation–SD) of the pigs at the start of the experiment was 22.7 kg (1.2) (Fig. 1). There was a significant increase in the bodyweights over the time in both groups increasing gradually until week 4. Although there was a slightly lower weight gain at week 5 in Group 1 – 44.8 kg (3.2) compared to Group 2 – 41.4 kg (4.3), the difference was not statistically significant.

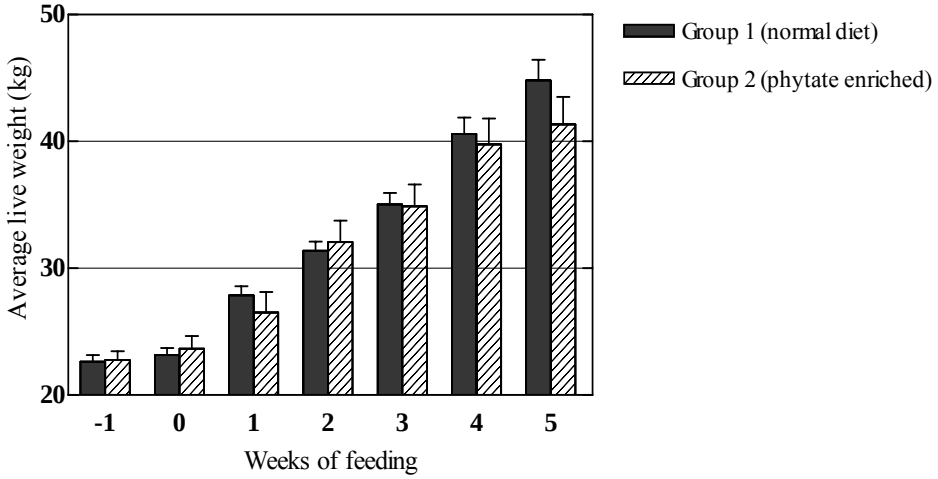

Figure 1. **Effect on growth of pigs of phytate-enriched and normal diets.**

The patterns of blood plasma zinc and AP concentrations of the pigs throughout the experiment are shown in Figs 2 and 3. Comparison of zinc concentrations revealed that at week 3 Group 2 exhibited a decrease which continued downward until week 5, when the mean (SD) level of zinc dropped to 9.2 μmol/l (2.2), compared with 14.1 μmol/l (1.9) in Group 1 (p=0.015).

The alkaline phosphatase levels exhibited a similar trend in zinc levels in pigs fed phytate (Fig. 3). The activity of alkaline phosphatase in Group 2, compared with Group 1 started to decrease from 3rd week and the difference between means at week 5 was markedly different; mean (SD) of alkaline phosphatase activity in Group 2 was 223 (u/l) (84.6) and that in Group 1 was 401 (u/l) (86.2)(p=0.025).

The liver zinc concentrations after 5 weeks (Fig. 4) demonstrated that pigs fed phytate (Group 2) had significantly lower mean (SD) levels of liver zinc - 78.7 ppm (4.5) compared to pigs in group 1- 101.0 ppm on a dry matter basis (18.4) (p<0.05).

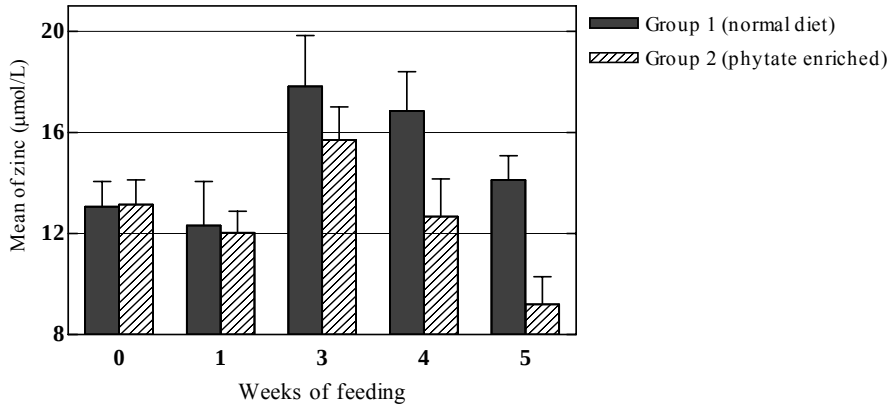

Figure 2. **Effect of dietary phytic acid on zinc in pigs blood plasma.**

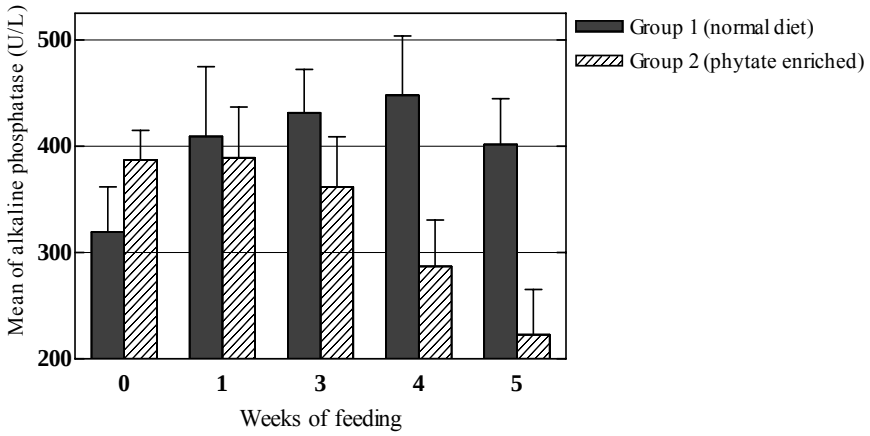

Figure 3. **Effect of dietary phytic acid on alkaline phosphatase in pigs blood plasma.**

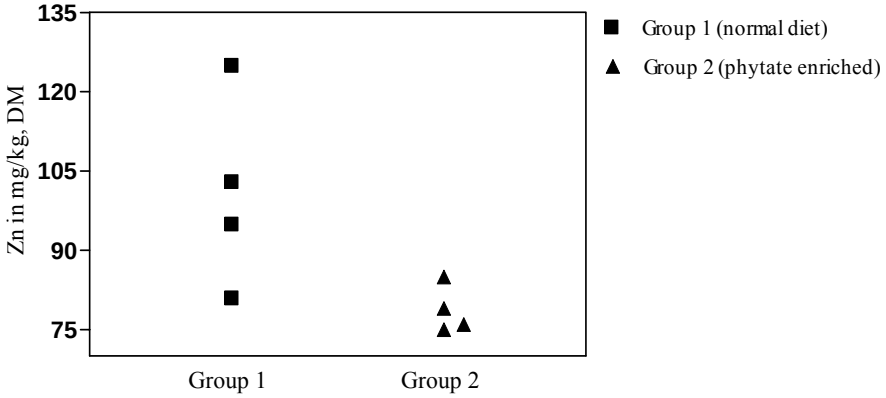

Figure 4. **Comparison of liver zinc levels in pigs on normal and phytate-enriched diets.**

Discussion. In this experiment we compared the influence of a diet enriched with phytate on the levels of Zn and alkaline phosphatase in blood plasma and liver of growing pigs. The results clearly demonstrated that the feeding of phytic acid at the levels we employed produced a state of zinc deficiency in the pigs between 3 and 5 weeks. The mechanism, by which phytate acts when ingested in an intrinsic or extrinsic form is well documented, i.e. a sequestering of metals (notably zinc) to the phosphorus groups in the compound, thereby reducing the intestinal uptake of the elements. The depriving effect of phytate was evident in the significant decrease of zinc and alkaline phosphatase in blood plasma and zinc deposition in liver. However, the phytate-rich diet did not affect significantly the growth rate of the pigs in the observed period. The decline of zinc and alkaline phosphatase in blood plasma occurred without overt clinical signs of zinc deficiency within the experimental period. The relevance of these parameters is supported by numerous studies, showing that blood plasma zinc level is a specific indicator and can be used for assessing zinc status in animals and men (Underwood, Suttle, 1999). Similarly, AP activity and zinc liver deposition reflect zinc status (Larsen, Sandstöm, 1993).

In conclusion, this experiment demonstrated that phytic acid significantly reduced the availability of dietary zinc and that it decreased circulating zinc and alkaline phosphatase in blood, thereby reducing zinc liver deposition and the general zinc status in growing pigs. Based on these results the feeding of phytic acid can be employed to create a zinc-deficient pig model for studies on the immune response of host to parasites and on parasite-host nutritional interactions.

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