Availability of microelements in recently weaned piglets fed diet supplemented with inulin

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ABSTRACT: The effects of inulin-supplemented diet on Cu, Mn, and Zn bioavailability in recently weaned piglets raised at an experimental and a commercial farm were studied. Piglets were weaned at 28 days of age, when all of them were fed a barley-wheat based starter diet supplemented with a usual vitamin-mineral premix for piglets. The reference diet group received additional 50 mg of zinc per kg diet, while the experimental inulin diet group was supplemented with 1.5% inulin (no additional zinc). Both diets, low in inorganic copper (provided only from forages), covered the demands for minerals of the growing piglets. Four piglets were sacrificed on days 28, 33, and 39 in each group at each farm and liver samples were collected and analyzed. The contents of Cu, Mn, and Zn were determined by flame atomic absorption spectrometry. The feeding experiments showed that the presence of inulin enhanced ($P < 0.05$) the biological availability of copper from the weaned piglets' diet despite its low content therein. No significant differences ($P > 0.05$) between experimental inulin diet group and reference diet group could be noticed for zinc and manganese concentrations in the liver. Biological availability of copper was higher in the piglets reared under high and standardized sanitary conditions than in those reared under commercial farm conditions ($P < 0.05$).

Keywords: liver; bioavailability; copper; manganese; zinc

The gastro-intestinal disorders that appear in the piglets shortly after weaning (such as diarrhoea) prompt the use of antibiotics and trace elements (e.g. copper, zinc) as growth promoters (Carlson et al., 1999; Lalles, 2008). Animal feeding and husbandry have to come up with alternative solutions to the use of in-feed antibiotics that have been banned in the European Union since 2006 (European Commission, 2003a, b). An attractive way of resolving the problem is to use natural substances like plant extracts or plant bioactive compounds in the diet under simultaneous body's mineral balance monitoring. Few studies evaluated effects of inulin supplementation on absorption of trace elements in monogastric farm animals (Yasuda et al., 2006). In experiments on rats, the presence of inulin in the diet reportedly increased the copper absorption (Coudray et al., 2006). It has been shown that bacterial fermentation of inulin-type fructans (ITF) in the large intestine was implicated in increased intestinal absorption and mineral bioavailability (Lobo et al., 2009a) of calcium, magnesium, and iron in rat and pig (Coudray et al., 2006; Yasuda et al., 2006; Lobo et al., 2009b). In contrast, studies of effects of ITF on absorption of micro-minerals (mainly copper, iron, and zinc) are relatively scarce and contradictory results have been obtained in experiments on rats (Scholz-Ahrens and Schrezenmeir, 2007). Molecular expression markers analysis revealed that inulin increased metabolic activity and reduced oxidative stress response in the mucosa of ileum of weaning piglets (Schwerin et al., 2009). Thus it can be supposed that feeding inulin could affect other epithelial physiological processes, e.g. transport of minerals. Moreover, different sanitary conditions were shown to affect the gene expression of the metabolic and immune markers (Schwerin et al., 2009).

Exact determination of trace elements content in the liver, the main storage organ of the organism, is of major importance in the studies on mineral availability from diets. It provides information on health status of the animal (Spears and Hansen, 2008). Few analytical data on cadmium, copper, lead, and zinc concentrations determined by both inductively coupled plasma mass spectroscopy and atomic absorption spectrometry in the digests of bovine liver reference material samples (Herber and Stoeppler, 1994; Zudowska and Biziuk, 2008; Gerber et al., 2009) provided evidence on suitability of the methods for studies on pigs.

This paper presents the results of feeding weaned piglets a diet supplemented with inulin on liver concentration of copper, manganese, and zinc. The experiment was performed parallelly at an experimental farm (EF) and a commercial farm (CF) in order to investigate possible effects of different sanitary regimes.

MATERIAL AND METHODS

Reagents

All reagents used were supplied by Merck (Darmstadt, Germany) and were of analytical purity. Concentrated aqueous solutions of 36% HCl (1.19 kg/l), 65% HNO_3 (1.39 kg/l), and 30% H_2O_2 were used. The reference material for the atomic absorption spectrometry (AAS) was CertiPUR® (Merck, Darmstadt, Germany). Inulin (I) extracted from chicory root (Raftiline® HP; BENEO-Orafti S.A., Tienen, Belgium) was used for the diet preparation.

Equipment

The following equipment was used: an atomic absorption spectrometer Solaar M6 Dual Zeeman Comfort (Thermo Electron Ltd., Cambridge, UK), with deuterium lamp for background correction and air-acetylene flame (Metrological certificate: 0170549/11.21.2007); a microwave digestion system with remote temperature measurement Speedwave MWS-2 Comfort (BERGHOF, Eningen, Germany); analytical balances (Sartorius AG, Göttingen, Germany); water distiller Milli-Q Ultrapure Water Purification System (Millipore, Billerica, USA); lyophilization device Gefriertrocknungsanlage TG 16 (VEB Hochvakuum, Dresden, Germany). Class A glassware was used for transvasation, dilution, and storage.

Microwave digestion method

Piglet liver samples of 0.4 g each were weighed with $\pm 2 \times 10^{-4}$ g accuracy. Each sample was processed as described previously (Untea et al., 2012) and a blank digest was carried out in the same way.

Flame atomic absorption spectrometry method

The samples of the reference diet and the experimental inulin diet as well as the liver samples were analyzed for copper, manganese, and zinc concentrations applying flame atomic absorption spectrometry (FAAS) after the microwave digestion. Working parameters: wave length (λ; in nm): 324.8 for Cu, 279.5 for Mn, and 213.9 for Zn; bandpass (in nm): 0.5, 0.2, and 0.5 for Cu, Mn and Zn, respectively; lamp current (in mA): 5 for Cu, 12 for Mn, and 10 for Zn.

Feeding experiment on piglets

The experiment was performed on a total of 24 male weaning piglets of German Landrace sows from the 2nd to the 6th parity, in two farms in parallel: one with environmental conditions common to pigs production (commercial farm (CF)) and the other with high and standardized sanitary conditions (experimental farm (EF), a part of the Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany) (Janczyk et al., 2009). To reduce the genetic difference between the litters, all piglets had one father boar. The groups of piglets were weight-balanced. The average initial weight of the piglets was 8.6 ± 1.7 kg. The piglets were weaned at 28 days of age, at either farm. Then four piglets at CF and other four at EF were sacrificed and livers were taken for analysis. The remaining piglets at each farm were allocated to two groups per 8 piglets: a control group – reference diet group (RD), which was offered a barley-wheat based starter diet supplemented with a usual vitamin-mineral premix for piglets (SD) (Schwerin et al., 2009) and additional 50 mg of zinc per kg diet, and an experimental inulin diet group (SD + I) fed the SD supplemented with 1.5% inulin (no additional zinc). The trace mineral-vitamin premix (0.4% both in the RD and SD + I groups) supplied vitamins (A 1750 IU, D3 200 IU, E 11 IU, K1 0.5 mg, B_1 1.0 mg, B_2 4 mg, D-pantothenic acid 9 mg, niacin 12.5 mg, biotin 50 µg, B_{12} 15 µg, folic acid 0.3 mg, B_6 1.5 mg, choline 400 mg), microelements (Fe 80 mg, Zn 54 mg, Mn 30 mg, Co 0.15 mg, I 0.14 mg, Se 0.25 mg), and antioxidants (E310, 320, 321) 50 mg per kg diet (Schwerin et al., 2009). The piglets were fed the respective diets twice daily, at 8.00 a.m. and 4.00 p.m., *ad libitum*. Water was supplied *ad libitum* via drinking nipples. Four animals from each group were sacrificed on days 33 and 39 of life, respectively, and the livers were collected. All piglets were weighed just before euthanasia.

Sample collection and storage

The liver weight was recorded immediately after dissection and an aliquot was snap frozen in liquid nitrogen and stored at –80°C until further processing. The frozen samples were lyophilized as follows: at 0°C for 12 h, 5°C for 12 h, and then at 20°C for 2 days. Dry matter content of each liver was then calculated. Two working samples were obtained from each liver sample for microwave digestion and FAAS determination.

Statistical analysis

The liver mineral concentrations were calculated on dry matter basis. The mineral concentrations in the liver samples from the piglets sacrificed on day 28 were used as a baseline for calculation of the differences in the concentrations on days 33 and 39 within and between groups. The analytical data were compared by the Analysis of Variance (ANOVA), using StatView for MS Windows (Statistical Analysis System, Version 6.0). The differences between mean values in the groups were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

In the present study, inulin was chosen both as a potential alternative to in-feed antibiotics and in the view of possible reduction of the level of the inorganic zinc and copper used as dietary growth promoters in the piglets' diets (European Commission, 2003a). Inulin in the experimental inulin diet was used in the concentration of 1.5%, because a similar concentration has been reported to have an effect on porcine gastrointestinal microbiota and short chain fatty acids concentration (Loh et al., 2006).

Mineral contents of the diet

Main minerals in the diets calculated on the fed basis were (in g/kg): Ca 7.2, total P 6.1, digestible P 3.65, Na 2.5, K 8.5. Trace elements determination of the SD showed that it contained (per kg diet): 6.44 mg Cu (only from dietary forages), 84.88 mg Zn (both from dietary forages and premix), and 37.44 mg Mn (both from dietary forages and premix), and so the diet covered the demands of the growing piglets (National Research Council, 1998; GfE, 2006). The diets used in the experiments were formulated to provide 12.5 g/kg of lysine and 10.0 MJ/kg of energy.

Minerals determination in the liver

The copper, manganese, and zinc concentrations in the piglet liver samples were determined as an indicator for measurement of the trace elements bioavailability (Richards et al., 2010) of the diet supplemented with inulin.

The copper, manganese, and zinc concentrations recorded in the liver samples from CF and EF are presented in Table 1. The values recorded for the investigated microelements remained in agreement with the literature reports (Leibholz et al., 1962; Apgar et al., 1995; Jondreville et al., 2005). The baseline of the data in Table 1 was the Cu, Mn, and Zn content in the liver of the piglets slaughtered at 28 days of age. No significant differences ($P >$ 0.05) were observed for liver zinc concentrations

Table 1. Mean values ± standard deviations of copper, zinc, and manganese concentrations* in the liver of weaned piglets fed with reference diet (RD) or experimental inulin diet (SD + I) and reared in experimental (EF) or commercial (CF) farm

Farm, age	RD			SD + I		
	Cu	Zn	Mn	Cu	Zn	Mn
EF						
Day 28 (n = 4)	266.24^{a} ± 10.5	232.42^{a} ± 7.1	9.37 ± 0.5	266.24^{a} ± 10.5	232.42^{a} ± 7.1	9.37 ± 0.5
Day 33 (n = 4)	163.68^{b,A} ± 4.6	183.77^{b} ± 15.9	9.03 ± 1.1	149.32^{b,B} ± 7.2	176.40^{b} ± 9.6	8.82 ± 0.9
Day 39 (n = 4)	184.76^{c,A} ± 5.7	137.39^{c} ± 19.1	9.54 ± 0.9	205.32^{c,B} ± 9.9	134.01^{c} ± 7.6	8.92 ± 1.1
CF						
Day 28 (n = 4)	207.14^{a} ± 5.7	206.79^{a} ± 8.7	10.41 ± 0.8	207.14^{a} ± 5.7	206.79^{a} ± 8.7	10.41 ± 0.8
Day 33 (n = 4)	144.12^{b} ± 16.5	193.43^{a} ± 17.1	10.85 ± 0.5	143.38^{b} ± 10.8	188.57^{b} ± 19.4	10.79 ± 0.6
Day 39 (n = 4)	26.62^{c,A} ± 5.5	131.95^{b} ± 2.8	10.29 ± 1.4	71.31^{c,B} ± 7.6	128.15^{c} ± 15.2	9.93 ± 1.4

n = number of piglets examined

*trace elements are expressed in mg/kg of liver tissue dry matter (mg/kg DM)

$^{a-c}$values with different superscripts differ significantly (P < 0.05) when comparing piglets of different age within the same diet group

^{A,B}values with different superscripts differ significantly (P < 0.05) when comparing piglets of the same age within different diet groups

between the dietary groups of piglets on days 33 and 39. However, in comparison to days 28 and 33, the liver Zn concentration decreased significantly on day 39 (P < 0.05), at the end of the experiment, when it represented 63.81% (RD) and 61.97% (SD + I) of the baseline concentration (at day 28). No effect of inulin on the liver Zn concentration could be noticed. The dietary manganese level (37.44 mg/kg) provided via the premix corresponded to the feeding requirements (Jondreville et al., 2005). Data in Table 1 show that the liver manganese concentration was constant throughout the experiment and did not differ (P > 0.05) between the RD and the SD + I groups. The inulin supplement seemed to have no effect on the dietary manganese and zinc absorption, in contrast to results of Coudray et al. (2006). For similar dietary concentrations of copper, provided only through the dietary forages, addition of inulin enhanced copper absorption and utilization. At CF, the liver copper concentration both in RD and SD + I was several times lower than the baseline at the end of the experiment (Table 1). Results show no significant differences for the copper concentrations between the two dietary groups after the first 5 days of treatment (day 33), but liver copper content was significantly higher (P = 0.0002) in the piglets from SD + I group on day 39. This finding indicates higher absorption of this mineral from gut in the presence of inulin in the diet. Differences between farms could be recorded, and comparison of the values obtained for both the EF and the CF group revealed that: (*i*) Cu concentration was higher in the liver of piglets from EF than at CF on days 28 and 39, and (*ii*) at either farm the Cu liver concentration was higher in SD + I than in RD on day 39 (Table 1). Low copper level in the liver can be a result of increased utilization or lower absorption, or combination of both. Copper deficiency determines a low activity of superoxide dismutase (SOD) in the liver and red cells (Iskandar et al., 2005). On the other hand, increased oxidative stress activates the response of SOD and copper utilization, what at least in part could explain the differences between the farms. In another study performed at the same farms, Schwerin et al. (2009) showed elevated mucosal immune response of animals kept at the CF. The lower sanitary conditions and higher microbial load from the environment could have resulted in activation of the immune system and higher utilization of copper in the piglets from CF. The immune responses were beyond the scope of this study and additional research would be needed to confirm this hypothesis.

The higher copper level in the livers from the piglets from the SD + I group in comparison to RD group indicates positive influence of inulin on the intestinal absorption of copper. Changes in intestinal microbial community and activity that were recorded in this study and published by

Janczyk et al. (2010) could have had an effect on the intestinal physiology and transport of copper. Moreover, the intestinal microbiota of the piglets from CF was beneficially affected by inulin supplementation in a more pronounced way than in the piglets from EF (Janczyk et al., 2010). This additionally supports the hypothesis that the lower sanitary conditions and thus higher microbial load at CF affected the immune and anti-oxidative stress response resulting in lowering of liver copper concentration. However, despite the low Cu concentrations at the end of the experiment, the copper contents in the liver of the piglets from both farms remained in agreement with the literature reports for piglets of the same age (Apgar et al., 1995; Jondreville et al., 2005).

CONCLUSION

Dietary inulin seems to increase relative biological availability of dietary copper in recently weaned piglets, without effects on zinc and manganese. Sanitary regimes may additionally affect the availability of dietary copper.

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