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SHORT COMMUNICATION

Diets enriched with chicory-derived native inulin can affect kidney and liver mineral content in nursery pigs

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ABSTRACT. Short-chain fatty acids originating from the gut microbiota are the main fermentation products of prebiotics and have recently emerged as key factors influencing the local absorption of dietary macro- and micronutrients. However, it is unclear whether such effects occur in growing pigs, as the existing results are contradictory. Therefore, this study aimed to assess the impact of including 1% (T1 diet) or 3% (T2 diet) native chicory inulin in the diet of nursery pigs on the mineral status of the liver and kidney. Feeding the T2 diet for 40 days, starting from day 10 after birth, increased the deposition (mg/kg) of Mg ($P < 0.05$), K ($P < 0.01$) and Se ($P < 0.01$) in the kidney compared to the control diet. It also increased Na ($P < 0.05$) and Se ($P < 0.01$) accumulation in the liver, while decreasing Zn concentration in the kidney ($P < 0.05$). Administration of both T1 and T2 diets for 40 days elevated Na ($P < 0.01$) content in the kidney and reduced Zn ($P < 0.01$) and Cd ($P < 0.05$) concentrations in the liver of pigs. In conclusion, it appears that inulin supplementation can increase Mg, K, Na and Se content in tissues. This finding is of particular importance due to the well-documented immunostimulatory and antioxidant properties of Se, as well as its capacity to chelate heavy metals such as Cd, thereby mitigating their toxicity. However, our results did not confirm any improvement of Zn content in the tissue.

Introduction

Chicory-derived inulin, a non-digestible carbohydrate, is widely recognised for its prebiotic properties, as confirmed by the International Scientific Association for Probiotics and Prebiotics (ISAPP) (Gibson et al., 2017). Fermentation of inulin by endogenous microbiota, including *Christensenellaceae*, *Actinobacillus Ruminococcus*, *Butyricicoccus*, *Roseburia*, *Lachnospiraceae* and *Lactobacillus*, results in the production of bacterial fermentation end products in the form of short-chain fatty acids (SCFA), such as acetate, propionate, and butyrate (Gardiner et al., 2020). These microbial SCFAs stimulate local absorption of dietary macro- and micronutrients by increasing their solubility and/or transcriptional activation of their specific transporters, thus increasing their accumulation in various tissues (Vanhoof and De Schrijver, 1996; Scholz-Ahrens and Schrezenmeir, 2007).

It remains unclear whether such effects are consistently observed in growing pigs, as current findings are contradictory. In our previous study (Lepczyński et al., 2021), dietary supplementation with 2% native chicory inulin or 4% chicory root improved the Ca and Se status of the liver and kidney in nursery pigs. However, similar effects regarding tissue mineral contents were not observed in weaning and growing pigs fed diets enriched with 3% or 6% inulin (Jolliff and Mahan, 2012).

Considering this context, this study aimed to evaluate the potential impact of a 40-day dietary supplementation with two different levels of native chicory inulin (1% or 3%) on tissue deposition of several minerals in healthy nursery pigs.

Material and methods

Experimental design and animal housing

The study was reviewed and approved by the Local Ethics Committee for the Care and Use of Laboratory Animals in Szczecin, Poland (protocol No. 13/2012 of 23/05/2012). The experiment involved 24 castrated PIC × Penarlan barrows divided into three groups (n = 8). The animals were fed for 40 days, beginning at 10 days of age, an unsupplemented cereal-based diet (C) or a diet enriched with 1% (T1) or 3% native chicory inulin (T2) (Inulin Orafti® GR, BENEO GmbH, Mannheim, Germany). The housing and feeding conditions were described in our previous work (Herosimczyk et al., 2020). Briefly, piglets were weaned after a four-week suckling period and offered solid diets as a mash placed in a feeder from day 10 of age. On day 28, two piglets from each litter (4 litters/group) were selected and distributed according to dietary treatment to separate pens (2 pens/group, 4 animals/pen) with free access to feed and water. After weaning, all diets were provided in the form of 4-mm diameter pellets. Feed intake and health status were monitored daily during the whole experiment. Animals were weighed at weaning and at the end of the experiment. After 40 days of feeding, pigs were euthanised by electrical stunning followed by exsanguination at 50 days of age and approximately 18 kg of body weight. The ingredient composition, nutrient and energy content of the diets, as well as the chemical composition of inulin are detailed in Tables 1 and 2, respectively. Table 3 lists mineral content of all diets. Following euthanasia, the left lateral lobe of the liver and kidneys were collected. The dissected tissue fragments were washed twice with 0.9% NaCl. Tissue samples were snap-frozen in liquid nitrogen directly after collection.

Table 1. Ingredients and chemical composition of pig diets

Item	Diets
Ingredients, %	
wheat	46.84
barley	20
maize starch[1]	3
full-fat soybean	5.90
whey	9.70
fish meal	4.00
spray-dried blood plasma	4.00
soybean oil	3.40
calcium formate	0.30
limestone	0.50
dicalcium phosphate	0.60
sodium chloride	0.07
L-lysine	0.61
DL-methionine	0.23
L-threonine	0.26
L-tryptophan	0.09
native chicory inulin[2]	±
aroma	0.10
mineral-vitamin premix[3]	0.40
Chemical composition, %	
dry matter	90.03
crude ash	4.54
crude protein	20.05
ether extract	6.04
crude fibre	1.52
fructan	1.00
ME, MJ/kg[4]	14.30

[1] inulin was added as a maize starch replacement (3, 2 or 0% of the total diet); [2] native chicory inulin (Inulin Orafti®GR, BENEO GmbH, Mannheim, Germany) containing approximately 92% inulin with DP ≥ 10 and 8% other carbohydrates (glucose, fructose and sucrose); inulin was added at 0, 1 or 3% of the total diet; [3] contained per kg: IU: vit. A 600 000, vit. D_3 60 000; mg: vit. E 3 000, vit. K_3 120, vit. B_1 120, vit. B_2 240, vit. B_6 240, nicotinic acid 1 600, pantothenic acid 800, folic acid 160, biotin 10, vit. B_{12} 1.6, I 40, Se 16, Co 16; g: choline chloride 12, Mg 3.2, Fe 6, Zn 5.6, Mn 2.4, Cu 6.4, I 40, Se 16, Co 16; [4] metabolisable energy, calculated values

Table 2. Chemical composition (%) of inulin used as a feed supplement

Item, %	Inulin[1]
Dry matter	92.59
Crude protein	0.16
Crude ash	0.02
Ether extract	0.05
Crude fibre	0.18
Fructans	92.00

[1] inulin Orafti®GR, BENEO GmbH, Mannheim, Germany (92% inulin with an average degree of polymerisation of 10, 8% simple sugars)

Tissue mineral composition analysis

The mineral composition (Mg, Ca, Na, K, Zn, Cu, Fe, Se, P, Al, Mn, Cr, Pb, Cd) of tissues (liver

Table 3. Macro- and micromineral content of pig diets: control diet (C), diets supplemented with 1% (T1) or 3% (T2) native chicory inulin and inulin used as a supplement, mg/kg

Mineral content,	Diet			Supplement
mg/kg	C	T1	T2	Inulin[1]
Mg	1885	1436	1482	0.640
Ca	11880	10240	14240	50.45
Na	2830	2727	3040	140.9
K	8270	6847	7207	27.55
Zn	231	230	286	N/D
Cu	32.40	31.06	37.67	N/D
Fe	559	478	597	0.334
Mn	158	152	182	0.044
Cr	1.698	1.170	1.292	N/D
Pb	2.63	2.61	1.92	N/D
Cd	0.178	0.190	0.150	N/D
Se	2.70	2.63	2.39	0.183
P	7970	7453	8040	N/D
Al	356	241	297	1.766

N/D – under detection limit; [1] inulin Orafti® GR, BENEO GmbH, Mannheim, Germany (92% inulin with an average degree of polymerisation of 10, 8% simple sugars)

and kidney) and feed (C, T1 and T2 diets) was determined using inductively coupled argon plasma optical emission spectrometry (ICP-OES) with a Perkin-Elmer Optima 2000 DV system. Approximately 0.5 g of each homogenised tissue and feed samples was transferred into a quartz vial containing a mineralising solution (5 ml of 65% HNO_3 and 0.5 ml of 35% H_2O_2 for tissues; 5 ml of 65% HNO_3, 0.5 ml of 35% H_2O_2 and 0.5 ml of HF for feeds) and subjected to microwave digestion in a sealed vessel. The solutions obtained after the mineralisation of the feed were complexed with 0.5 g of H_3BO_3 to bind the fluoride ions remaining in the post-reaction solution Mineralisation was conducted in an Anton Paar Multiwave microwave oven (Anton Paar Ltd., Hertford, UK) using a four-phase program: for the first 5 min, the power gradient ranged from 100 to 600 W; from minute 6 to 10, it was maintained at 600 W; from minute 11 to 20, it was held at 1000 W or less depending on critical values (75 MPa or 300 °C); from minute 21 to 35, the vials were cooled. After cooling, the digested solutions were transferred into flasks and diluted with deionised water to a final volume of 10 ml. The a fore mentioned mineral components were determined in the prepared solutions using standard, certified multi-element ICP solutions: Multi-element Standard IV, Multi-element Standard VIII and Multi-element Standard XIV (Merck, Darmstadt, Germany). The quality control of the ICP-OES method was evaluated by analysing a reference tissue sample (bovine muscle 8414; NIST, Gaithersburg, MA, USA), and the results are given in Table 4.

Table 4. Bovine muscle reference material (RM 8414) analysis

Element	Concentration of elements in reference material 8414, mg/kg				Fold change: observed result/certified result
	Certified result	1st replicate	2nd replicate	observed result	
Pb	0.38	0.367116	0.409929	0.388522	1.022
Cd	0.013	0.014316	0.014184	0.01425	1.096
Zn	142	137.0659	153.1915	145.1287	1.022
Cu	2.84	2.80652	2.893617	2.850069	1.004
Mn	0.37	0.357193	0.38156	0.369377	0.998
Cr	0.071	0.067612	0.069504	0.068558	0.966
Fe	71.2	67.32814	69.64539	68.48676	0.962
Al	1.7	1.672573	1.531915	1.602244	0.942
Ca	145	147.4132	151.7731	149.5931	1.032
Se	0.076	0.07197	0.07311	0.07254	0.954
K	15170	15450.04	15602.84	15526.44	1.023
Na	2100	2026.931	2000	2013.466	0.959
Mg	960	944.011	968.794	956.403	0.996
P	8360	8532.955	8964.539	8748.747	1.047

Statistical analysis

Statistical analysis were performed using the SAS 9.3 Package (SAS Institute Inc., Cary, NC, USA). Mean values and standard error of the mean (SEM) were calculated. Tissue mineral analysis data were analysed using one-way analysis of variance followed by Fisher's test based on the following linear model:

$$y_{ij} = \mu + \alpha_i + e_{ij},$$

where: y_{ij} – the individual observation, μ – the overall mean, α_i – the treatment effect and e_{ij} – the residual error. A *P*-value of less than 0.05 was considered statistically significant.

Results

Production traits

All animals remained healthy showing no signs of diarrhoea throughout the experimental period. Supplementing the piglets' diets with 1% or 3% native chicory inulin did not significantly affect their body weight (BW) at any time point, nor their final BW. These results were previously reported in a study by Herosimczyk et al. (2020) and are summarised in Table 5. Feed intake was not analysed statistically, as it was only estimated on a per-pen basis.

Table 5. Mean body weight (BW) and BW gain in pigs fed a control diet (C) and diets supplemented with 1% (T1) or 3% (T2) native chicory inulin

Parameters	Diet			SEM	*P*-value
	C	T1	T2		
BW at weaning, kg	8.00	7.30	7.50	0.29	0.6576
Final BW, kg	17.90	16.70	17.50	0.50	0.6222
BW gain, kg	9.90	9.40	10.00	0.35	0.7339

SEM – standard error of the mean; $P > 0.05$

Tissue mineral composition

The mineral composition of the liver and kidney of pigs is presented in Table 6. In the liver, feeding both T1 and T2 diets significantly ($P < 0.05$) decreased Cd concentrations compared to the control group, whereas the T2 diet decreased Zn content compared to the other groups ($P < 0.01$). The T1 diet significantly increased P content in relation to the other groups ($P < 0.05$), while Na and Se levels were higher ($P < 0.05$) in pigs on the T2 diet than in those administered the T1 and C diets. There was no effect recorded of varying inulin levels on Mg, Ca, K, Cu, Fe, Al, Mn, Cr, and Pb concentrations.

In the kidney of pigs fed the C and T1 diets, Mg, K, and Se concentrations were lower compared to those on the T2 diet ($P < 0.05$, $P < 0.01$, and $P < 0.01$, respectively). Both the T1 and T2 diets increased Na levels compared to the C group ($P < 0.01$). Dietary inulin level also affected Zn and Mn concentrations; the former was lower and the latter higher in pigs receiving the T2 diet compared to those given the C and T1 diets, respectively ($P < 0.05$). However, different inulin levels in the diet did not affect concentrations of Ca, Cu, Fe, P, Al, Cr, Pb, and Cd in the kidney of pigs.

Table 6. Liver and kidney mineral content of nursery pigs fed a cereal-based control diet (C) and diets enriched with 1% (T1) or 3% (T2) native chicory inulin

Mineral content	Groups			SEM	*P*-value
	C (n = 8)	T1 (n = 8)	T2 (n = 8)		
Liver, mg/kg wet tissue					
Mg	194.36	201.57	200.38	1.912	0.2659
Ca	44.57	45.07	44.49	0.946	0.9679
Na	789.13^{a}	788.37^{a}	887.65^{b}	19.019	**0.0416**
K	3248.50	3249.22	3392.50	34.599	0.1493
Zn	82.24^{b}	75.61^{a}	49.27^{a}	3.944	**0.0002**
Cu	18.11	21.08	21.45	1.297	0.5346
Fe	71.14	89.28	85.60	8.215	0.6547
Se	0.212^{a}	0.313^{a}	0.724^{b}	0.0778	**0.0105**
P	3102.18^{a}	3302.97^{b}	3094.51^{a}	38.772	**0.0370**
Al	0.654	0.598	0.520	0.0996	0.8692
Mn	3.153	3.433	3.455	0.0673	0.1215
Cr	0.034	0.031	0.034	0.0017	0.6683
Pb	0.195	0.197	0.202	0.0024	0.4152
Cd	0.021^{b}	0.019^{a}	0.020^{a}	0.00004	**0.0126**
Kidney, mg/kg wet tissue					
Mg	185.23^{a}	187.44^{a}	197.79^{b}	1.976	**0.0159**
Ca	74.40	71.12	75.59	1.702	0.5504
Na	1198.14^{a}	1271.45^{b}	1328.07^{b}	17.174	**0.0043**
K	2919.18^{a}	2853.39^{a}	3129.34^{b}	38.417	**0.0037**
Zn	25.44^{b}	22.35^{ab}	20.52^{a}	0.787	**0.0325**
Cu	9.17	7.82	6.84	0.535	0.2259
Fe	20.95	21.41	24.12	1.580	0.7061
Se	0.484^{a}	0.448^{a}	0.799^{b}	0.0529	**0.0060**
P	2574.47	2477.73	2519.09	28.451	0.3931
Al	0.653	0.654	1.578	0.2108	0.1218
Mn	1.506^{ab}	1.412^{a}	1.713^{b}	0.0488	**0.0274**
Cr	0.022	0.023	0.031	0.0028	0.4099
Pb	0.0079	0.0079	0.0084	0.00012	0.0816
Cd	0.021	0.020	0.021	0.0003	0.2573

SEM – standard error of the mean; $P > 0.05$; ^{ab} means within rows with different superent scripts are significantly different at $P < 0.05$; bold values indicate statistical significance at $P < 0.05$

Discussion

The results of the current study clearly demonstrated that the T2 diet had more pronounced effects on mineral concentrations in the kidney and liver. Dietary supplementation with 3% inulin appeared to increase Se content in both liver and kidney, with its levels increasing nearly 4-fold and 2-fold, respectively. Se is known for its immunoprotective and antioxidant effects and has demonstrated the ability to interact with heavy metals, forming complexes that prevent their toxic effects on the body (Jan et al., 2015). These insoluble Se-metal complexes are more readily excreted in the urine, and thus excluded from further biochemical transformations. These findings

were further supported by the significant decrease in Cd concentration in the liver of pigs fed both experimental diets. Emerging evidence suggests that supplementing the diet of weanling pigs with high levels of Zn can effectively reduce post-weaning diarrhoea, while also improving average daily gain, average daily feed intake, feed efficiency, and final body weight gain (Wei et al., 2020; Hutchens et al. 2021). Hutchens et al. (2021) discussed various mechanisms that may underlie these beneficial effects of Zn. For instance, high dietary Zn levels have been shown to stimulate ghrelin secretion, thereby increasing feed intake and nutrient digestibility. Additionally, Zn reinforces intestinal barrier function by promoting the expression of tight junction proteins, which reduces intestinal permeability and prevents the translocation of pathogenic bacteria, ultimately lowering the incidence of diarrhoea (Hutchens et al., 2021). Interestingly, we have found that diets enriched with inulin do not improve tissue Zn content in nursery pigs. This outcome contrasts with the observations of Vanhoof and De Schrijver (1996), who noted a tendency for higher faecal and ileal absorption, as well as increased Zn retention in pigs fed wheat- and barley-based diets containing 6% native chicory inulin compared to control animals. However, the present result is consistent with our earlier study (Lepczyński et al., 2021), where we observed a statistically significant decrease in Zn content in the liver and kidney of nursery pigs fed diets enriched with 2% native chicory inulin or 4% dried chicory root. The absorption of trace elements, particularly Fe, Cu and Zn, from inulin-supplemented diet can be affected by several factors. These include the composition of the basal diet, the degree of polymerisation (DP) of the fructans and the dose (Scholz-Ahrens and Schrezenmeir, 2007). The main difference between the current results and those of Vanhoof and De Schrijver (1996) presumably lies in the dosage, which was twice as high in their study compared to ours. This difference likely induced distinct caecal and colonic fermentation patterns. Specifically, the higher dose of 6% inulin tended to decrease luminal pH, creating a more acidic environment, a crucial factor known to increase the solubility and absorption of trace minerals. In contrast, our previous study (Barszcz et al., 2018), conducted on the same group of animals, found that dietary native inulin at levels of 1%, 2%, or 3% did not affect the pH of digesta in the caecum, proximal colon, and distal colon. However, in the middle colon, pigs fed a diet enriched with 1% inulin showed a higher pH of digesta compared to the control group. The degree of polymerisation (DP), which refers to the number of individual fructose units in the inulin chain, may also be a key factor affecting its intestinal stability. Various studies have indicated that the DP of inulin determines its predominant fermentation site. For instance, Loh et al. (2006) found that 20–50% native inulin (DP 10) was fermented in the jejunum of pigs, while Paßlack et al. (2012) observed that the proximal colon was the primary fermentation site of long-chain inulin (DP 57). These variations in fermentation patterns can lead to differences in SCFA production and, consequently, to a potential pH decrease in different segments of the gut. This may subsequently affect the solubility of minerals and their absorption in the intestines. The differences observed across studies, including variations in the number of animals used, and the specific time of inulin application, contribute to the discrepancies in the reported outcomes. The findings of Jolliff and Mahan (2012) further corroborate this conclusion. Their research, conducted on weanling and growing pigs fed with 3% and 6% native chicory inulin (for weanling pigs) or 6% native inulin (for growing pigs) in maize and soybean meal did not reveal significant changes in kidney or liver mineral concentrations. Interestingly, our study also demonstrated an increased accumulation of renal Mg in the T2 group. This finding aligns with our former study (Robak et al., 2016), where supplementation with 2% native inulin was found to induce increased expression of the TRPM6 protein. TRPM6 forms a Mg-permeable ion channel located in the proximal and distal tubule of the renal cortex, and in the collecting ducts of the renal medulla, mediating Mg intake from the tubular lumen.

Conclusions

Our findings suggest that a 40-day feeding period with a diet containing 3% native chicory inulin (T2 diet) significantly increased Se bioavailability, resulting in a marked increase in its levels in both the liver and kidneys of nursery pigs. This finding is particularly interesting given the immune-enhancing and antioxidant properties of Se, as well as its ability to bind to heavy metals such as Cd. However, contrary to prior research, we did not observe any increase in tissue Zn content. Additionally, increased renal Mg accumulation was observed in the T2 group, which was consistent with our previous findings indicating that inulin facilitates renal Mg uptake.

Conflicts of interest

The Authors declare that there is no conflict of interest.

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