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ORIGINAL PAPER

EFFECTS OF INULIN EXTRACTS AND INULIN-CONTAINING PLANTS ON HAEMATOBIOCHEMICAL RESPONSES, PLASMA MINERAL CONCENTRATIONS, AND CARCASS TRAITS IN GROWING-FINISHING PIGS*

Wioletta Samolińska[1], Eugeniusz R. Grela[1], Bożena Kiczorowska[2]

[1] Sub-Department of Bromatology and Nutrition Physiology
[2] Sub-Department of Animal Nutrition and Feed Science
University of Life Sciences in Lublin, Poland

Abstract

The aim of the present study was to assess the effects of dietary supplementation with extracts of differently polymerised inulin and inulin-containing plants (Jerusalem artichoke, chicory) on selected slaughter parameters in swine as well as on their blood profile associated with the mineral status of an organism and the functional status of the liver. The experiment was carried out throughout an entire fattening period and involved 120 pigs with the initial weight of 30.0 ± 0.5 kg. The pigs were divided into 5 groups. The dietary treatments consisted of control diet (CON) and control diet supplemented with extracts of inulin (EI) – standard inulin (SI; average degree of polymerisation ≥ 10) or long-chain inulin (LCI, average degree of polymerization ≥ 23), and control diet supplemented with inulin-containing plants (ICP): Jerusalem artichoke (JA) or chicory (CH). The overall inulin content in the treatments was similar (20 g inulin kg^{-1} diet). The ICP treatments increased the iron, zinc, and copper content in pig blood plasma and the mean corpuscular haemoglobin concentration. They also reduced the activity of liver enzymes in blood plasma, compared with the control. In the EI treatments, an increase in the plasma iron concentration and the MCHC value and a decrease in the relative liver weight and stabilisation of the activity of liver enzymes in relation to the control were noted. The effect of inulin supplementation was more pronounced when the compound was added to the diet in the first fattening stage.

Keywords: inulin, pigs, haematology, biochemical indices, plasma minerals, carcass traits.

Wioletta Samolińska, PhD, Sub-Department of Bromatology and Nutrition Physiology, Institute of Animal Nutrition and Bromatology, University of Life Sciences, Akademicka 13, 20-950 Lublin, Poland, e-mail: wioletta.samolinska@up.lublin.pl, orcid.org/0000-0002-8999-1036

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INTRODUCTION

Phytobiotics, i.e. plant-based formulations exerting a wide spectrum of health-enhancing effects on the organism and simultaneously enhancing production performance, are currently gaining popularity in animal production. An example is inulin, i.e. a polysaccharide with prebiotic properties extracted from such plants as Jerusalem artichoke or chicory and exerting a positive effect on the gastrointestinal microbiome. However, there are still only few reports on the other biological activity of the compound, e.g. its effect on the mineral status of the organism or its hepatoprotective activity.

Inulin is a long-chain fructan composed of multiple β-D-fructose units linked by a β-(2.1) glycosidic bond with a terminal glucose molecule in the chain. It is characterised by varied degrees of polymerisation (DP) ranging from 2 to ca. 65 units, with an average degree of polymerisation $DP_{av} = 12$ (Kelly 2008). The length of the chain has an impact on the prebiotic and technological properties of inulin (Tárrega et al. 2011). Inulin is often extracted from plants accumulating large quantities of fructans as storage material in roots and rhizomes in, e.g. chicory (*Cichorium intybus* L.), Jerusalem artichoke (*Helianthus tuberosus* L.), dandelion (*Taraxacum officinale* (L.) Weber), and other species. In the gastrointestinal tract, inulin serves as a hydrolysis and fermentation substrate for the beneficial intestinal microbiota and increases the abundance of bacteria, primarily from the genus *Bifidobacterium* (Gibson 1998) and some *Lactobacillus* species (Kaplan, Hutkins 2000, Han et al. 2014). Additionally, inulin contributes to enhancement of the bioavailability of mineral compounds in the gastrointestinal tract, which is associated with the production of SCFA – short chain fatty acids (Coudray et al. 2006, Yasuda et al. 2006, Lobo et al. 2009). Retention of mineral components in the organism is determined by the supply and excretion thereof. In the case of the supply, the content of an element consumed in the diet and its bioavailability are essential. Therefore, there is an intensified search for methods to enhance absorption of "deficit" elements from animal and human diet. One of the nutritional strategies involves additional supplementation of organic-bound elements and the use of functional additives increasing utilization thereof directly from food. Blood serum and plasma are frequently tested for assessment of the normal element supply of the organism. The content of elements in the organism is a result of a dynamic balance between the demand and the amount supplied, and the capability of elimination thereof (Reinhold 1975). Inulin is also regarded as an additive with hepatoprotective properties effects through stabilisation of liver function (Kim, Han 2013). There are available results of research of the prebiotic activity of inulin in fattener feeding (Varley et al. 2010, Vhile et al. 2012, Sobolewska et al. 2014, Grela et al. 2016), whereas there are only scarce reports on other biological properties of the compound, e.g. enhancement of the bioavailability of elements, impact on the mineral

status of the organism (Jayasooriya et al. 2009, Vhile et al. 2012, Samolińska, Grela 2017), or hepatoprotective effect in this technological group of pigs.

The aim of the investigations was to assess the effects of dietary supplementation with extracts of inulin characterised by different degrees of polymerisation and inulin-containing plants (Jerusalem artichoke, chicory) on selected slaughter parameters in pigs as well as their haematological and biochemical blood profile associated with the mineral status of the organism and the functional status of the liver.

MATERIALS AND METHODS

Ethical statement

The methodology used in the present study was approved by the Local Ethics Committee on Animal Experimentation of the University of Life Sciences in Lublin, Poland.

Experimental design, dietary treatments, and animal management

The experiment was conducted on 120 crossbreed pigs (Polish Landrace x x Large White) x Duroc with the initial weight of 30.0 ± 0.5 kg; the pigs were divided into 5 equinumerous groups (24 pigs per group). Four animals were kept per pen (two gilts + two barrows). Each pen was equipped with a stainless steel self-feeder and a nipple drinker. The pigs had free access to feed and drinking water throughout the experimental period. The fatteners were fed *ad libitum* with complete diets, i.e. grower (30-70 kg) and finisher (71-115 kg). The hygienic conditions, i.e. the temperature, relative humidity, and cooling were the same for all the groups.

The dietary treatments consisted of control diet (CON), control diet supplemented with extracts of inulin (EI) – standard inulin (SI) or long-chain inulin (LCI), and control diet supplemented with inulin-containing plants (ICP) Jerusalem artichoke (JA) or chicory (CH). The inulin content in the diets was similar in each treatment (20 g inulin kg^{-1} diet). In the SI, LCI, JA and CH diets, it was calculated based on the manufacturer's declaration (Beneo-Orafti, Tienen, Belgium; Vitamix, Niemcz, Poland). The inulin extracts from chicory (*Cichorium intybus* L.) roots used in the experiment differed in the degree of polymerisation (DP), i.e. standard inulin (SI, Orafti® GR, DP_{av} of inulin ≥ 10) or long-chain inulin (LCI, Orafti® HPX, DP_{av} of inulin ≥ 23). The inulin-containing plants were added to the diets in the form of fragmented dried tubers (Jerusalem artichoke) or root powder (chicory). Corn starch replaced the inulin extracts and inulin-containing plants in the control diet. The feed mixtures comprised grain meal (barley and wheat), soybean meal, soybean oil, and mineral feeds (monocalcium phosphate, limestone ground, and mineral-vitamins premix). The isocaloric

and isonitrogenous diets were balanced for metabolisable energy and nutrients. The composition and chemical analysis of the diets are presented in Table 1.

Carcass performance

During the experiment, the animals were weighed at the start and before slaughter. Feed intake was controlled individually by weighing portions for automatic feeding in the pens. On experimental day 98, six pigs from each group (three gilts and three barrows), weighing approximately 115 kg, were slaughtered. The slaughter was conducted in accordance with the technology currently employed in the meat industry with the use of electrical stunning. The carcass traits were estimated according to the following formulas (CARPENTER et al. 2004):

$$\text{Carcass daily gain (kg day}^{-1}\text{)} = ((\text{carcass weight} - (\text{initial live weight} \times 0.65))/\text{days to slaughter}$$

Carcass feed conversion ratio = total feed intake/total carcass gain.

At the slaughter operations, the livers were individually weighed. Relative liver weight was calculated as g organ kg^{-1} live weight.

Sample collection and chemical analyses

The diets were analysed for total protein content (AOAC, 2000). The lysine content was determined using method 994.12 (AOAC 2000), whereas cysteine and methionine were determined according to SCHRAM et al. (1954).

Samples of the mixtures were dried at 100°C for 24 h and ashed for 10 h at 550°C. The ashed samples were dissolved in a nitric acid-perchloric acid mixture (1:1) and diluted with deionized water for mineral analysis. The Ca, Mg, Fe, Zn, and Cu contents were measured using flame atomic absorption spectrophotometry (FAAS) (Unicam 939/959AA-6300, Shimadzu Corp., Tokyo, Japan) according to the Polish Standard (PN-EN ISO 6869, Polish Standard, 2002) and the total P content was determined colorimetrically (PN-76/R-64781, Polish Standard, 1976) with a Helios Alpha UV-VIS apparatus (Spectronic Unicam, Leeds, United Kingdom). Calcium was determined at λ = 422.7 nm, magnesium at λ = 285.2 nm, zinc at λ = 213.9 nm, copper at λ = 324.8 nm, iron at λ = 248.3 nm, manganese at λ = 279.5 nm, and phospohorus at λ = 400 nm. Ca and Mg were analysed by addition of 0.4% w/v of lanthanum oxide (Merck, Poland), which is a correction buffer that allows binding of the analysed element to the matrix.

Blood samples were taken from the external jugular vein of six clinically healthy animals (three gilts and three barrows) fasted overnight for 8 h, twice in each group, when the body weight of the animals was 60 and 100 kg. Blood samples were collected for the haematological analyses in 2-ml Vacutest tubes with K_3EDTA anticoagulant (Vacutest Kima s.r.l., Arzergrande (PD), Italy). For biochemical assays, blood was sampled in 6-ml Vacutest

Table 1

Ingredients and chemical composition of the experimental diets (as feed basis)

Item	Diets*									
	Grower (30-70 kg)					Finisher (71-115 kg)				
	CON	extracts of inulin		inulin--containing plants		CON	extracts of inulin		inulin--containing plants	
		SI	LCI	JA	CH		SI	LCI	JA	CH
Ingredients (g kg^{-1})										
Barley	370	370	370	370	370	482	481	481	481	481
Wheat	300	300	300	300	300	300	300	300	300	300
Soybean meal	242	242	242	242	242	150	150	150	150	150
Corn starch	40	20	20	-	-	40	20	20	-	-
Soybean oil	12	12	12	12	12	-	-	-	-	-
Mineral-vitamins premix**	20	20	20	20	20	15	15	15	15	15
Standard inulin	-	20	-	-	-	-	20	-	-	-
Long-chain inulin	-	-	20	-	-	-	-	20	-	-
Dried Jerusalem artichoke tubers	-	-	-	40	-	-	-	-	40	-
Chicory root powder	-	-	-	-	40	-	-	-	-	40
Monocalcium phosphate	4	4	4	4	4	3	3	3	3	3
Limestone ground	12	12	12	12	12	11	11	11	11	11
Analysed chemical composition (g kg^{-1})										
ME (MJ kg^{-1})***	12.93	12.86	12.86	12.58	12.58	12.64	12.46	12.46	12.28	12.28
Crude protein	175.46	175.27	175.27	175.36	175.36	156.2	155.1	155.0	154.2	153.3
Lysine	10.81	10.76	10.75	10.77	10.78	8.84	8.81	8.81	8.78	8.79
Methionine+cysteine	5.82	5.81	5.79	5.83	5.82	5.43	5.40	5.41	5.39	5.41
Total calcium	7.12	7.11	7.11	7.13	7.14	6.45	6.44	6.43	6.46	6.46
Total magnesium	2.11	2.13	2.14	2.19	2.12	1.40	1.42	1.42	1.47	1.42
Total phosphorus	5.07	5.05	5.05	5.09	5.08	4.89	4.87	4.87	4.88	4.85
Total iron (mg)	95.78	94.61	94.55	99.83	94.23	87.95	88.54	88.57	89.92	87.71
Total zinc (mg)	87.38	86.41	86.41	87.80	86.59	79.01	78.59	78.86	79.19	78.81
Total copper (mg)	9.99	9.91	9.83	10.02	9.94	8.83	8.75	8.76	8.82	8.78

* Pigs were fed diets differing in the source of inulin as follows: CON – control diet without inulin supplementation, SI – control diet with supplementation of standard inulin (Orafti® G, Beneo-Orafti, Tienen, Belgium, contained ~92% inulin, ~8% glucose, fructose, and sucrose [solubility < 12 g l^{-1} and DP ≥ 10]), LCI – control diet with supplementation of long-chain inulin (Orafti® HPX, Beneo-Orafti, Tienen, Belgium, containing 100% inulin [solubility < 5 g l^{-1} and DP ≥ 23]), JA – control diet with supplementation of dried Jerusalem artichoke tubers (containing ~49% inulin), CH – control diet with supplementation of chicory root powder (contained ~52% inulin).

** One kg of the mineral-vitamins premix containing vitamin A 600,000 IU, D_3 60 000 IU, E 3000 mg, K_3 120 mg, B_1 120 mg, B_2 240 mg, B_6 240 mg, B_{12} 1.6 mg, nicotinic acid 1600 mg, pantothenic acid 800 mg, folic acid 160 mg, biotine 10 mg, choline chloride 12 g, Mg 12.5 g, Fe 1.5 g, Zn 4 g, Mn 3.5 g, Cu 100 mg, I 75 mg, Se 20 mg, Co 25 mg.

*** Metabolisable energy was calculated according to the equation proposed by Kirchgessner and Roth (1983).

tubes containing lithium heparin (Vacutest Kima s.r.l., Arzergrande (PD), Italy).

Whole blood was analysed within three hours after sampling. After placement of the samples on a haematological mixer (UMH-5, Wigo, Pruszkow, Poland), the haematological parameters were determined using a haematological analyser ABACUS Junior Vet (Automatic cell counter, Diatron, Vienna, Austria) with specific software for pig porcine blood samples. The parameters analysed included the number of red blood cells (RBC), haemoglobin concentration (HGB), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), and packed cell volume (PCV).

Plasma was obtained for the analysis of biochemical parameters by centrifugation of whole blood at 3000 rpm (603 x g) for 15 min in a laboratory centrifuge (MPW-350R, MPW Medical Instruments, Warsaw, Poland) at a temperature of 4°C. Plasma without haemolysis signs was analysed within four hours after sampling and the calcium, phosphorus, magnesium, iron, zinc, and copper contents were determined. The elements were determined in blood plasma with colorimetric methods according to the manufacturer's protocol using reagent kits (BioMaxima, Lublin, Poland; Hydrex Diagnostics, Warsaw, Poland) and a random access biochemical analyser Metrolab 2300 GL (Metrolab SA, Buenos Aires, Argentine). The activity of alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) was analysed as well. The blood parameters were determined in blood plasma with colorimetric methods according to the manufacturer's protocol using reagent kits (BioMaxima, Lublin, Poland; Hydrex Diagnostics, Warsaw, Poland) and a random access biochemical analyser Metrolab 2300 GL. The analysis procedures were verified with the use of multiparametric control plasma (BioCal), as well as control plasma with a normal level (BioNorm) and a high level (BioPath) of blood parameters (BioMaxima, Lublin, Poland; Hydrex Diagnostics, Warsaw, Poland).

Statistical analysis

The statistical analysis was performed using software package Statistica version 13. The normality and homogeneity of variances were tested using the Shapiro-Wilk and Levene tests, respectively. Data were presented as means and pooled standard error means (SEM). The effects of the dietary inulin supplementation on all variables were analysed with one-way analysis of variance using the GLM procedure (ANOVA; Fisher test or Welch test):

$$Yij = \mu + a_i + e_{ij},$$

where: Yij – the measured variable, μ – an overall mean; ai – the dietary inulin effect (treatment), and eij, – the random error.

Means were compared using the *post-hoc* Duncan's test. All statements of significance are based on a probability < 0.05.

To determine the effect of the different sources of inulin, the data were analysed using a contrast to compare all sources of inulin treatments with the controls according to the following model: (1) the difference between the control (CON) and the treatments with inulin supplementation (SI + LCI + + JA + CH)/4; (2) the difference between the control (CON) and the treatments with supplementation inulin extracts (SI + LCI)/2; (3) the difference between the control (CON) and the treatments with supplementation of an inulin-containing plant (JA + CH)/2; (4) the difference between the inulin extract treatments (SI + LCI)/2 and the treatments with supplementation of an inulin-containing plants (JA + CH)/2.

RESULTS AND DISCUSSION

Carcass traits

In the present study, there was no modifying effect of the inulin addition to pigs' diets on the carcass weight, carcass daily gain, or carcass feed conversion ratio (Table 2). This is in line with results reported in other studies. In investigations conducted by Grela et al. (2013), 3% inulin supplementation in mixtures did not have an impact on carcass quality traits of fattening pigs. Similarly, Vhile et al. (2012) did not find an effect of Jerusalem artichoke addition to diets for male pigs on carcass traits.

In turn, the inclusion of inulin extracts, especially long-chain inulin, was associated with reduced relative liver weight (one-way ANOVA; CON vs EI; $P < 0.05$) – Table 2. The available literature provides reports of the activity of inulin in enhancement of liver function and the hepatoprotective properties of the compound. Products of prebiotic fermentation (SCFA) can influence the expression of genes encoding liver enzymes involved in the regulation of energy metabolism and increase the expression of proteins involved in protection of hepatocytes against oxidative stress (den Besten et al. 2013, Lepczyński et al. 2017). Results reported by Okada et al. (2017) indicate that the intake of *Helianthus tuberosus* tuber powder inhibits the accumulation of fat and glycogen in the liver. This positive effect of inulin on the liver can explain the differences in the weight of this organ.

Plasma mineral concentrations

In the present study, the blood parameters were determined in the reference ranges for this animal species reported in available literature (Weiss, Wardrop 2010, Reece 2015). It was found that inulin treatments exerted a more distinct effect on plasma mineral concentrations in the first stage of fattening (Table 3) than in the finishing stage (Table 4). The highest iron concentration in blood plasma of growing pigs was found in the JA and LCI treatments (one-way ANOVA, $P < 0.001$), in comparison with the control

Table 2

Effect of inulin on pig carcass traits

Variables	Treatments*					Pooled SEM	*P* value ANOVA	Inulin supplementation	Source of inulin		*P* value contrast**			
	CON	extracts of inulin		inulin-containing plants										
		SI	LCI	JA	CH			I	EI	ICP	CON vs I	CON vs EI	CON vs ICP	EI vs ICP
Carcass weight (kg)	89.53	89.12	89.89	89.28	88.93	0.152	0.851	89.31	89.51	89.11	0.796	0.711	0.865	0.808
Carcass daily gain (kg day^{-1})	0.715	0.710	0.718	0.712	0.708	0.003	0.521	0.712	0.714	0.710	0. 913	0.743	0.915	0.826
Carcass feed conversion ratio	3.21	3.23	3.18	3.21	3.26	0.014	0.795	3.22	3.21	3.24	0.861	0.724	0. 783	0. 837
Relative liver weight (g kg^{-1})	15.63^{a}	15.12^{ab}	14.68^{b}	15.89^{a}	15.22^{ab}	0.120	0.035	15.23	14.90	15.56	0.934	0.043	0.941	0.072

Data are the means from 6 pigs (three barrows and three gilts) per group, SEM – standard error of the means, *a*, *b* – …statistical differences $P < 0.05$.
* Treatments: CON – control, SI – standard inulin, LCI – long-chain inulin, JA – dried Jerusalem artichoke tubers, CH – chicory root powder.
** Contrast: (CON vs I) difference between the control and treatments with inulin supplementation (SI + LCI + JA + CH)/4, (CON vs EI) difference between the control and treatments with supplementation with the inulin extracts (SI + LCI)/2, (CON vs ICP) difference between the control and treatments with supplementation with the inulin-containing plants (JA + CH)/2, (EI vs ICP) difference between the inulin extract treatments (SI + LCI)/2 and treatments with supplementation with the inulin-containing plants (JA + CH)/2.

Table 3

Effect of inulin on the plasma mineral concentrations, haematological indices, and enzyme activity in the blood of growing pigs

Variables		Treatments*					Pooled SEM	*P* value ANOVA	Inulin supplementation	Source of inulin		*P* value contrast**			
		CON	extracts of inulin		inulin-containing plants										
			SI	LCI	JA	CH			I	EI	ICP	CON vs I	CON vs EI	CON vs ICP	EI vs ICP
Plasma mineral concentrations	calcium (mmol l^{-1})	2.33	2.23	2.32	2.36	2.52	0.04	0.151	2.36	2.28	2.44	0.754	0.592	0.288	0.068
	phosphorus (mmol l^{-1})	2.34	2.40	2.59	2.49	2.50	0.05	0.650	2.49	2.50	2.49	0.327	0.323	0.425	0.836
	magnesium (mmol l^{-1})	0.77	0.73	0.86	0.74	0.87	0.02	0.065	0.80	0.79	0.81	0.526	0.551	0.578	0.977
	iron (µmol l^{-1})	17.98^{b}	16.58^{b}	22.50^{a}	26.00^{a}	16.75^{b}	0.91	<0.001	20.46	19.54	21.38	0.021	0.049	0.008	0.089
	zinc (µmol l^{-1})	11.98^{b}	12.05^{b}	15.10^{ab}	16.90^{a}	15.73^{a}	0.54	0.001	14.94	13.58	16.31	0.003	0.103	<0.001	0.003
	cooper (µmol l^{-1})	26.4^{ab}	24.60^{b}	29.33^{ab}	30.52^{a}	31.31^{a}	0.79	0.015	28.94	26.96	30.91	0.133	0.745	0.024	0.018
Haematological indices	RBC (10^{12} l^{-1})	7.25	6.81	7.11	6.83	7.13	0.10	0.548	6.97	6.96	6.98	0.322	0.318	0.420	0.833
	HGB (mmol l^{-1})	7.36	7.34	7.25	7.23	7.67	0.07	0.250	7.37	7.29	7.45	0.924	0.728	0.603	0.295
	MCV (fl)	52.72	52.81	47.42	49.90	52.99	0.99	0.306	50.78	50.12	51.44	0.429	0.335	0.632	0.543
	MCH (pg)	16.38	17.45	16.45	17.08	17.32	0.21	0.374	17.08	16.95	17.20	0.198	0.331	0.167	0.592
	MCHC (g l^{-1})	310.48^{b}	330.52^{ab}	348.05^{a}	345.24^{a}	326.71^{b}	4.96	0.021	337.63	339.28	335.97	0.024	0.028	0.048	0.738
	PCV (l l^{-1})	0.38	0.36	0.34	0.34	0.38	0.01	0.060	0.35	0.35	0.36	0.076	0.097	0.088	0.307
Activity of enzymes in the blood plasma (U l^{-1})	ALP	202.35	176.25	177.55	172.75	229.50	8.97	0.212	189.01	176.90	201.13	0.509	0.299	0.872	0.303
	ALT	40.37	34.64	43.15	36.70	40.78	2.23	0.800	38.82	38.89	38.74	0.748	0.829	0.715	0.851
	AST	47.89^{a}	35.05^{b}	45.77^{ab}	43.74^{ab}	41.11^{ab}	2.09	0.039	41.42	40.41	42.43	0.749	0.244	0.558	0.490

Data are the means from 6 pigs (three barrows and three gilts) per group, SEM – standard error of the means, *a*, *b* – …statistical differences $P < 0.05$.

* Treatments: CON – control, SI – standard inulin, LCI – long-chain inulin, JA – dried Jerusalem artichoke tubers, CH – chicory root powder.

** Contrast: (CON vs I) difference between the control and treatments with inulin supplementation (SI + LCI + JA + CH)/4, (CON vs EI) difference between the control and treatments with supplementation with the inulin extracts (SI + LCI)/2, (CON vs ICP) difference between the control and treatments with supplementation with the inulin-containing plants (JA + CH)/2, (EI vs ICP) difference between the inulin extract treatments (SI + LCI)/2 and treatments with supplementation with the inulin-containing plants (JA + CH)/2.

RBC – red blood cells, HGB – haemoglobin, MCV – mean corpuscular volume, MCH – mean corpuscular haemoglobin, MCHC – mean corpuscular haemoglobin concentration, PCV – packed cell volume, ALP – alkaline phosphatase, ALT – alanine aminotransferase, AST – aspartate aminotransferase.

treatment (by 45% and 25%, respectively) – Table 3. A similar effect of inulin was recorded in the case of zinc as well. The highest concentrations, increased by 41% and 31%, were found for the JA and CH treatments, respectively (one-way ANOVA, $P = 0.001$). The contrast analysis confirmed the significant effect of the inulin treatments on the concentration of trace minerals in the plasma of growing pigs. In comparison with the control, the inulin (I) addition to the mixtures, regardless of its source, increased the blood iron concentration by 14% (CON vs I; $P < 0.05$), by 9% in the treatments with the inulin extracts (CON vs EI; $P < 0.05$), and by 19% in the treatments with the inulin-containing plants (CON vs ICP; $P < 0.01$). The blood plasma zinc concentration increased by 25% upon the inulin treatments (CON vs I; $P < 0.05$). The ICP treatments induced a significantly higher increase (by 36%) than the CON treatment ($P < 0.001$) and EI (by 20%) ($P < 0.01$). Additionally, the contrast analysis revealed an increase in the copper content in the blood of growing pigs induced by the ICP treatments, in comparison with the CON treatment (by 17%) $P < 0.05$ and EI (by 15%) – $P < 0.05$. In the second stage of fattening, the changes in the plasma mineral concentrations induced by the inulin addition to the diets were less pronounced (Table 4). The highest iron concentration of all the treatments was noted in the case of the JA application. The contrast analysis also showed a significant effect of the EI treatments on the increase in the plasma zinc concentration in finishing pigs, compared with the control (by 13%) (CON vs EI; $P < 0.05$) and the other inulin source (by 11%) (EI vs ICP; $P < 0.05$).

Similarly, other authors reported a stimulating effect of inulin, which was reflected by an increase in Fe, Zn, and Cu absorption in the gastrointestinal tract and by an increase in the concentration of the elements in the plasma of various livestock or laboratory animals (Lopez et al. 2000, Lobo et al. 2009, Tiengtam et al. 2015, Coudray et al. 2016, Samolińska, Grela, 2017). In a study conducted by Lepczyński et al. (2016), the level of plasma iron in piglets fed diets supplemented with dried chicory root was increased. The mechanism of the rise in the availability of macro- and micronutrients induced by inulin is multidirectional. It is associated with modulation of the intestinal microbiome, primarily by enhancement of the growth of the genus *Bifidobacterium* and some *Lactobacillus* species. As a substrate for hydrolysis and fermentation carried out by the desirable microbiome, inulin stimulates the production of organic acids (mainly SCFA), which reduce the pH of intestinal contents, thereby increasing the solubility of mineral components (Gibson 1998, Scholz-Ahrens, Schrezenmeir 2007, Han et al. 2014). Enhanced formation of SCFA indirectly improves absorption of minerals by stimulating the proliferation of epithelial cells aimed at enlargement of the absorptive surface area as well as alleviating inflammation and improving the intestinal epithelium integrity, which protects against pathogenic bacteria and potentially stimulates the expression of mineral-transport proteins in epithelial cells (Scholz-Ahrens, Schrezenmeir 2007).

Table 4

Effect of inulin on the plasma mineral concentrations, haematological indices, and enzyme activity in the blood of finishing pigs

Variables		Treatments*					Pooled SEM	*P* value ANOVA	Inulin supplementation	Source of inulin		*P* value contrast**			
		CON	extracts of inulin		inulin-containing plants										
			SI	LCI	JA	CH			I	EI	ICP	CON vs I	CON v s EI	CON vs ICP	EI vs ICP
Plasma mineral concentrations	calcium (mmol l^{-1})	2.48	2.52	2.40	2.49	2.42	0.02	0.281	2.46	2.46	2.45	0.646	0.727	0.625	0.864
	phosphorus (mmol l^{-1})	2.64	2.60	2.51	2.55	2.37	0.04	0.019	2.51	2.56	2.46	0.334	0.929	0.075	0.097
	magnesium (mmol l^{-1})	0.79	0.81	0.85	0.77	0.80	0.02	0.701	0.81	0.83	0.79	0.724	0.440	0.893	0.272
	iron (µmol l^{-1})	17.50^{ab}	17.15^{ab}	16.93^{ab}	19.45^{a}	15.50^{b}	0.38	0.006	17.26	17.04	17.48	0.724	0.543	0.974	0.482
	zinc (µmol l^{-1})	16.90	19.95	18.30	15.43	18.80	0.49	0.017	18.12	19.13	17.11	0.220	0.050	0.841	0.032
	cooper (µmol l^{-1})	35.07	37.33	39.13	41.91	36.02	0.94	0.140	38.60	38.23	38.96	0.118	0.195	0.115	0.705
Haematological indices	RBC (10^{12} l^{-1})	7.07	7.03	6.81	6.25	6.69	0.17	0.575	6.70	6.92	6.47	0.399	0.761	0.221	0.255
	HGB (mmol l^{-1})	7.76	8.36	7.63	8.11	7.62	0.11	0.121	7.93	8.00	7.86	0.914	0.754	0.610	0.320
	MCV (fl)	52.49	56.90	51.63	60.58	61.83	2.01	0.408	57.74	54.26	61.21	0.310	0.750	0.132	0.141
	MCH (pg)	17.71	19.18	16.69	19.79	20.39	0.63	0.347	19.01	17.93	20.09	0.412	0.895	0.180	0.140
	MCHC (g l^{-1})	337.01	336.78	333.47	326.67	331.21	2.68	0.431	332.03	335.13	328.94	0.281	0.362	0.288	0.846
	PCV (l l^{-1})	0.37	0.40	0.36	0.39	37.0	0.01	0.089	0.39	0.38	0.39	0.153	0.534	0.072	0.092
Activity of enzymes in the blood plasma (U l^{-1})	ALP	195.60^{a}	198.73^{a}	157.48^{b}	157.60^{b}	141.30^{b}	7.38	0.025	163.78	178.10	149.45	0.046	0.292	0.011	0.045
	ALT	56.88	52.80	54.59	51.09	51.29	1.59	0.799	52.44	53.69	51.19	0.313	0.505	0.241	0.520
	AST	76.18	64.41	70.65	61.03	68.26	2.00	0.236	66.09	67.53	64.65	0.041	0.101	0.034	0.487

Note: see in Table 3.

Haematological and biochemical indices

Assessment of the red blood cell indices facilitates determination of the status of iron and other erythropoietic elements in the organism. The effect of the inulin treatments on the haematological indices was recorded only in the growing period (Table 3). An increased MCHC value was found in the LCI and JA treatments, compared with the control treatment (respectively, by 12% and 11%) (one-way ANOVA, $P < 0.05$). The contrast analysis confirmed the significant effect of the inulin supplementation, irrespective of its source (CON vs. I) and related to the source (CON vs. EI; CON vs. ICP), on the increase in this haematological parameter ($P < 0.05$). These changes can be explained by the increased levels of elements involved in erythropoietic processes (iron, zinc, and copper). A similar effect of inulin on red blood cell indices was reported by YASUDA et al. (2006). They observed an increase in the haemoglobin level in piglets fed diets supplemented with a combination of long-chain inulin and oligofructose. In turn, TIENGTAM et al. (2017) noted that dietary supplementation with inulin (2.5 or 5.0 g kg^{-1}) and Jerusalem artichoke (5.0 or 10.0 g kg^{-1}) resulted in a significant increase in the RBC count in the Nile tilapia ($P < 0.05$). The authors explained this positive effect of inulin on the red blood cell system by higher assimilability of iron resulting from optimisation of intestinal conditions and greater effectiveness of utilisation of this element in the haemoglobin synthesis process.

The ALT, AST and ALP enzymes are used for evaluation of liver function. The ALP enzyme present in the membrane of various cells is involved in e.g. intestinal lipid transport and bone calcification. Plasma mainly contains ALP isoforms from bones and liver. Increased activity of alkaline phosphatase is observed in disorders of bone growth and calcification. In turn, the AST enzyme is produced in the liver as well as other tissues, including cardiac and skeletal muscle tissues. The elevation of its blood level indicates damage to cellular structures and is proportional to the degree of the damage (HOFFMANN, SOLTER 2008). The analysis of the activity of selected metabolic profile enzymes involved in liver function revealed a reduced rate of enzymatic reaction of alkaline phosphatase and aspartate aminotransferase upon inulin supplementation of the diets (Tables 3, 4). The effect was stronger in treatments where inulin was administered with dried Jerusalem artichoke or chicory. The correlation was evident mainly in the second fattening stage, i.e. after nearly 90 days of administration of the inulin-supplemented diets. In the growing pigs, the SI treatments reduced the AST activity (one-way ANOVA, $P < 0.05$) by 27%, in comparison with the control treatment (Table 3). In the second fattening stage, there was a decrease in the ALP activity in the LCI, JA, and CH treatments by 19%, 19%, and 28%, respectively, compared with the control (one-way ANOVA, $P < 0.05$). The contrast analysis demonstrated reduction of the ALP activity ($P < 0.05$) caused by the addition of inulin (CON vs. I) and the ICP treatments (CON vs. ICP). The comparison of the effect of two inulin sources (EI vs. ICP) on the ALP activity confirmed its reduction induced by the ICP treatments ($P < 0.05$). Similarly, reduction

of the AST activity caused by inulin supplementation was noted in the finishing pigs (CON vs I; $P < 0.05$) with a greater effect exerted by the ICP treatments (CON vs ICP; $P < 0.05$). There are many available studies showing the hepatoprotective effect of inulin. For instance, through positive modulation of the composition of the intestinal microbiota, inulin can suppress the negative effects of metabolic disorders induced by pathogens and chemicals on the liver (Cani et al. 2013, Reygner et al. 2016). In turn, the study carried out by Corrêa-Ferreira et al. (2017) showed that inulin from *Artemisia vulgaris* supplementation (10 mg kg^{-1}) in mice diet exerted considerable hepatoprotective effects *in vivo*, which can be attributed to antioxidant and immunomodulatory properties, as suggested by the authors. Similar results were obtained by Liu et al. (2015) at a higher dose of inulin from dahlia tubers – 400 mg kg^{-1} diet. In the present study, the effect of the supplementation with the inulin-containing plants (Jerusalem artichoke or chicory) on ALP and AST activity was more potent than that of the inulin extracts, which may be related to the richer chemical composition, including antioxidant compounds, in the plants (Jurgoński et al. 2011, Yang et al. 2015).

CONCLUSIONS

The research results showed a more pronounced effect of the supplementation with inulin originating from different sources on the iron, zinc, and copper status in pig blood plasma and on the mean corpuscular haemoglobin concentration in the first fattening period, in comparison with non-supplemented animals. The addition of dried Jerusalem artichoke tubers and chicory roots as an inulin source exerted more potent effects than the inulin extracts with different polymerisation degrees on the increase in the zinc and copper concentrations in blood plasma. The inulin treatments did not modify the carcass weight, carcass daily gain, or carcass feed conversion ratio. However, the dietary inulin inclusion had an impact on liver function by stabilisation of its enzymatic activity. In the case of supplementation with the inulin extracts, the liver was reduced, which was particularly evident after the application of the long-chain inulin.

In conclusion, given the beneficial effects of supplementation of growing--finishing pigs' diets with Jerusalem artichoke tubers as an inulin source on the plasma mineral status, supplementation with dried Jerusalem artichoke at a dose of 40 g kg^{-1} mixture (20 g inulin kg^{-1} mixture) can be recommended for the fattening process.

Conflict of Interest Statement

The authors declare that they have no conflicts of interest.

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