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Original article

Effect of inulin extracts or inulin-containing plant supplement on blood lipid indices and fatty acid profile in fattener tissues

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Abstract

The objective of the study was to evaluate the effect of inulin or dandelion, chicory and Jerusalem artichoke powder on lipid indices and fatty acid profile in fattener tissues. The experiment involved 120 crossbred pigs (PL × PLW) × Duroc with an initial body weight of 25.0 ± 0.5 kg. Animals were assigned into 6 groups. A diet for group I (control) did not comprise an inulin additive, group II and III received 2% inulin (water or water-alcohol extraction of inulin from chicory root, respectively), while the mixtures for group IV, V and VI contained 4% root powder from Jerusalem artichoke (topinambur), chicory or dandelion, respectively. The animals were slaughtered at 115 kg body weight. The samples of blood, liver and the muscle *longissimus dorsi* tissues as well as whole hearts were collected for analysis. Fatty acid profile, some lipid indices and crude fat and cholesterol content were evaluated. Dietary supplement of 40 g dandelion powder resulted in preferable significant changes in the blood lipid indices and fatty acids composition (increased PUFA share and decreased n-6/n-3 ratio).

Key words: inulin, finishing pigs, tissue, fatty acid profile, blood, lipid indices

Introduction

Lipid profile of blood, tissues and organs is associated with fat metabolism in organism conditioned by, among others, animal nutrition strategies such as feed fat content and fat type as well as some feed additives (Grela 1992, Otten et al. 1993, Bertin et al. 1994, Fernández et al. 2007). Withdrawal of the antibiotic growth promoters in animal diets resulted in intensive research on the alternative feed supplements, like probiotics, organic acids, herbs and prebiotics (Grela et al. 2011). Among the prebiotics, oligosaccharides deserve special attention, including inulin and other fructooligosaccharides (Gibson and Delzenne 2008, Kelly 2008, 2009, Nowak et al. 2012). Due to their chemical structure, these compounds are indigestible by human and monogastric animal enzymes in the upper gastrointestinal tract and thus, reach the large intestine unaltered. In the large bowel, they get disintegrated to short-chain fatty acids

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(propionic, lactic, etc.), which improves metabolism and overall animal health status (Roberfroid 2005, Yan et al. 2012). Some researches, though, highlight the contribution of inulin or inulin-storing plants to human health enhancement (Williams and Jackson 2002), improved animal body conditions (Beylot 2005), increased HDL-cholesterol content (Cieślik et al. 2005) and finally, immune response modulating effects (Delgado et al. 2010). Beneficial influence of oligosaccharides on bacterial flora and formation of short chain fatty acids in the large intestine are likely to induce changes in lipid metabolism in pig organism.

The objective of the present study was to assess the effectiveness of feed additive of entire inulin-containing plant powder and inulin obtained by water or water-alcohol extraction from chicory root on blood lipid profile indices and fatty acid fractions, including the atherogenicity index and thrombogenicity index as well as hypocholesterolemic/hypercholesterolemic acid ratio in backfat, *longissimus* muscle, heart and liver.

Materials and Methods

All the experimental procedures were approved by the Local Ethics Commission of the Faculty of Biology and Animal Breeding at University of Life Sciences in Lublin, Poland.

The experiment involved 120 crossbred pigs (PL × PLW) × Duroc with an initial body weight (BW) of 25.0 ± 0.5 kg. Animals were assigned to 6 groups (20 units, each), kept in pens (4 pigs per pen). Fatteners were fed the following mixtures – grower (25-70 kg BW) and finisher (71-115 kg BW). The diets comprised ground grain (wheat, barley and corn), soybean meal, soybean oil, mineral feeds (salt, monocalcium phosphate and ground limestone) and mineral-vitamin premix. The feeds were balanced for metabolizable energy, total protein, amino acid composition, minerals and vitamins (Grela et al. 2009). Diet for group I (control) did not incorporate an inulin additive, group II and III received 2% of inulin in mixture (water or water-alcohol extraction of inulin from chicory root, respectively). The mixtures for group IV, V and VI contained 4% root powder from topinambur, chicory or dandelion respectively. Since the content of inulin in the aforementioned powders is about 50%, it corresponds to the 2% content of inulin for groups II and III. All the animals had free access to feeders (*ad libitum* consumption) and drinkers. The hygienic conditions, that is, temperature, relative humidity and cooling were the same for all the groups. Inulin was extracted according to the modified Stahl and Schild method (1981), while water extraction and water-alcohol extraction (30% ethyl alcohol) was performed from chicory root.

Blood samples were taken from 8 animals from each group twice, at 50 kg and 100 kg BW. Blood plasma analyzed to determine triacylglycerols, total cholesterol, high-density-lipoprotein cholesterol fraction (HDL) was assayed colorimetrically using Biomaxima monotests and Metrolab biochemistry analyzer. Low-density-lipoprotein cholesterol (LDL) was estimated from the Friedewald et al. (1972) equation.

The pigs were slaughtered at about 115 kg BW. The slaughter was conducted in accordance with the technology currently employed in meat industry, using the electrical stunning. At slaughter operations, the heart and liver were weighed, whereas samples for the laboratory evaluation were collected from the liver, *longissimus dorsi* muscle, backfat and whole heart. The *longissimus dorsi* muscle (loin) samples were taken near the last thoracic and first lumbar vertebra, while backfat samples over the shoulder blade by cutting out a lobe of 5 cm width and 10 cm length from a forequarter cut. Immediately after collection, the samples were stored at -20°C temperature.

Total fat of backfat, liver, heart and the m. *longissimus dorsi* for fatty acid analysis was extracted with a chloroform/methanol mixture according to the Folch et al. (1957) method. Further investigations concerning fatty acid profile were conducted according to the following standards: PN EN ISO 5509:2001 and PN EN ISO 5508:1996. A percentage of fatty acid methyl esters was estimated using the gas chromatography procedure on a Varian CP-3800 chromatograph. The chromatograph operating conditions for fatty acid separation were as follows: the capillary column CP WAX 52CB DF 0.25 mm of 60 m length, gas carrier – helium, flow rate 1.4 ml/min, column temperature 120°C gradually increasing by 2°C/min up to 210°C, determination time 127 min, feeder temperature 160°C, detector temperature 160°C, other gases – hydrogen and oxygen. Cholesterol content in organs and tissues was measured using the colorimetric method of Rhee et al. (1982). Lipid quality indices, i.e. atherogenicity index (AI) and thrombogenicity index (TI) were calculated according to the Ulbricht and Southgate (1991) equations.

All the data were analyzed with the STATISTICA Software Ver. 6.1 (StatSoft 2003). The data were evaluated statistically using a general linear model (GLM) of analysis of one-way variance ANOVA. Duncan's test was applied for the multiple comparisons between means, considering $P \leq 0.05$ and $P \leq 0.01$ as significant. The tables illustrate the means, the standard error of means and the levels of significance (P value).

Table 1. Some lipid indices in blood of growing-finishing pigs.

Ingredients	BW, kg	Feeding groups						SEM	P value
		I	II	III	IV	V	VI		
Total cholesterol, mmol l^{-1}	50	2.35^{a}	2.24^{ab}	2.13^{b}	2.23^{ab}	2.15^{b}	1.93^{c}	0.09	0.036
	100	2.53^{a}	2.47^{a}	2.36^{ab}	2.32^{b}	2.29^{b}	2.19^{b}	0.08	0.044
HDL, mmol l^{-1}	50	0.86	0.88	0.82	0.88	0.87	0.86	0.03	0.233
	100	0.61^{ab}	0.64^{ab}	0.58^{b}	0.62^{ab}	0.63^{ab}	0.68^{a}	0.02	0.045
LDL, mmol l^{-1}	50	1.33^{a}	1.25^{a}	1.20^{ab}	1.25^{a}	1.14^{b}	0.95^{c}	0.04	0.031
	100	1.73^{a}	1.61^{ab}	1.58^{ab}	1.51^{b}	1.59^{ab}	1.34^{c}	0.05	0.041
TG, mmol l^{-1}	50	0.34^{a}	0.25^{b}	0.26^{b}	0.22^{b}	0.30^{ab}	0.26^{b}	0.02	0.045
	100	0.45	0.49	0.43	0.41	0.42	0.38	0.03	0.091
CHL/HDL	50	2.75^{a}	2.55^{b}	2.60^{ab}	2.53^{b}	2.66^{ab}	2.25^{c}	0.09	0.032
	100	4.19^{a}	3.85^{ab}	4.06^{a}	3.73^{b}	4.06^{a}	3.20^{c}	0.08	0.018
% HDL	50	36.4^{b}	39.2^{ab}	38.4^{ab}	39.6^{ab}	37.7^{b}	44.4^{a}	2.46	0.014
	100	23.9^{b}	25.9^{b}	24.6^{b}	26.8^{b}	24.6^{b}	31.2^{a}	1.86	0.025

$^{a, b, c}$– values in the same rows with different letters differ significantly ($p \leq 0.05$)

Table 2. Crude fat and cholesterol content in tissues and organs of finishing pigs.

Item	Feeding groups						SEM	P value
	I	II	II	IV	V	VI		
Muscle *longissimus:*								
– crude fat, %	2.49	2.38	2.34	2.39	2.41	2.31	0.12	0.096
– cholesterol, mg g^{-1}	0.61^{a}	0.55^{ab}	0.53^{ab}	0.58^{a}	0.54^{ab}	0.49^{b}	0.06	0.034
Liver*:*								
– crude fat, %	5.86	5.75	5.92	5.69	5.71	5.57	0.15	0.084
– cholesterol, mg g^{-1}	3.38^{a}	3.25^{ab}	3.19^{ab}	3.23^{ab}	3.15^{ab}	3.08^{b}	0.19	0.041
Heart*:*								
– crude fat, %	3.58	3.42	3.39	3.40	3.35	3.33	0.11	0.058
– cholesterol, mg g^{-1}	1.37	1.29	1.25	1.19	1.24	1.18	0.08	0.056
Backfat*:*								
– crude fat, %	83.15^{a}	82.11^{ab}	82.09^{ab}	81.15^{b}	81.12^{b}	80.83^{b}	0.85	0.039
– cholesterol, mg g^{-1}	1.16^{a}	1.07^{ab}	1.01^{b}	1.06^{ab}	1.11^{ab}	1.01^{b}	0.03	0.042

$^{a, b}$ – values in the same rows with different letters differ significantly ($p \leq 0.05$)

Results

The supplementation of fatteners' diet with inulin obtained by water-alcohol extraction (group III) and root powder from chicory (group V) or dandelion (group VI) has decreased total cholesterol level in blood plasma (Table 1). The most beneficial changes in blood lipid profile were noted in group VI (dandelion root feed additive), where the highest share of HDL-cholesterol fraction for both fattening periods under study was shown.

The included feed additives did not have any significant influence on a crude fat content in the liver, heart and m. *longissimus dorsi* (Table 2). Only in the backfat of animals from the groups fed plant powder-enriched diet a lower fat level was shown as compared to the control. Notably significant effect on cholesterol concentration in backfat, m. *longissimus dorsi* and liver was determined especially in the animals from group VI.

Fatteners' diet supplementation with inulin obtained by two different methods (group II and III) did not affect significantly a fatty acid profile in the fat of backfat (Table 3). The feed additives supplied to fatteners' diet were found to contribute to significant changes in n-6/n-3 acid ratio (Table 3). Far more variable fatty acid composition was established in the *longissimus* muscle, heart and liver. The most beneficial effect on fatty acid profile, AI, TI indices and h/H ratio appeared to be caused by dandelion root powder (group VI) and obtained differences in fat content in m. *longissimus dorsi* (Table 4), liver (Table 5) and heart (Table 6) between the control and group VI were statistically confirmed. The research results of

Table 3. Fatty acid composition (% total fatty acid) in backfat of finishing pigs.

Fatty acids	Feeding groups						SEM	P value
	I	II	III	IV	V	VI		
SFA	39.51^{b}	40.98^{ab}	40.83^{ab}	42.11^{a}	41.02^{ab}	40.21^{b}	1.21	0.015
MUFA	46.43	45.61	45.65	45.08	46.08	46.35	1.17	0.141
PUFA	12.77	12.38	12.58	11.75	11.49	12.01	0.34	0.058
Σ n-6/Σ n-3	13.03^{a}	12.60^{ab}	11.71^{b}	10.52^{c}	11.91^{b}	11.26^{bc}	0.17	0.043
AI	0.49	0.55	0.52	0.55	0.52	0.51	0.02	0.055
TI	1.21^{b}	1.30^{ab}	1.28^{ab}	1.35^{a}	1.32^{a}	1.27^{ab}	0.17	0.047
h/H	2.19	1.99	2.11	1.99	2.08	2.10	0.04	0.063

$^{a, b, c}$ – values in the same rows with different letters differ significantly (p≤0.05)

Table 4. Fatty acid composition (% total fatty acid) in fat of *longissimus dorsi* muscle.

Fatty acids	Feeding groups						SEM	P value
	I	II	III	IV	V	VI		
SFA	40.19^{a}	39.97^{a}	40.33^{a}	39.90^{a}	39.65^{a}	37.55^{b}	0.52	0.046
MUFA	48.40^{b}	48.39^{b}	48.56^{b}	50.06^{a}	49.67^{ab}	50.34^{a}	0.63	0.042
PUFA	10.65^{b}	10.87^{b}	10.56	9.92^{c}	10.56^{b}	11.82^{a}	0.22	0.041
Σ n-6/Σ n-3	16.75^{a}	15.72^{a}	16.03^{a}	16.10^{a}	16.60^{a}	12.43^{b}	1.15	0.011
AI	0.56	0.56	0.56	0.55	0.54	0.50	0.02	0.121
TI	1.29^{a}	1.27^{a}	1.29^{a}	1.26^{a}	1.24^{a}	1.12^{b}	0.04	0.045
h/H	1.91^{b}	1.91^{b}	1.90^{b}	1.95^{ab}	1.98^{ab}	2.13^{a}	0.07	0.039

$^{a, b, c}$ – values in the same rows with different letters differ significantly (p≤0.05)

Table 5. Fatty acid composition (% total fatty acid) in fat of pig liver.

Fatty acids	Feeding groups						SEM	P value
	I	II	III	IV	V	VI		
SFA	42.16^{a}	41.58^{ab}	41.02^{ab}	40.92^{ab}	40.25^{b}	39.12^{c}	1.47	0.044
MUFA	16.55	16.43	16.80	17.31	17.18	17.68	0.56	0.071
PUFA	38.71^{b}	38.92^{b}	39.06^{b}	38.88^{b}	39.53^{ab}	40.82^{a}	1.36	0.042
Σ n-6/Σ n-3	9.00^{a}	8.90^{a}	8.69^{ab}	8.41^{ab}	8.07^{b}	7.65^{b}	0.25	0.034
AI	0.32	0.32	0.32	0.32	0.31	0.29	0.02	0.063
TI	1.12^{a}	1.10^{a}	1.07^{ab}	1.06^{ab}	1.02^{ab}	0.94^{b}	0.07	0.044
h/H	3.15^{b}	3.16^{b}	3.17^{b}	3.19^{b}	3.33^{ab}	3.56^{a}	0.18	0.031

$^{a, b, c}$ – values in the same rows with different letters differ significantly (p≤0.05).

Table 6. Fatty acid composition (% total fatty acid) in fat of pig heart.

Fatty acids	Feeding groups						SEM	P value
	I	II	III	IV	V	VI		
SFA	32.67^{a}	32.11^{ab}	32.04^{ab}	31.76^{b}	32.05^{ab}	30.39^{c}	1.42	0.046
MUFA	25.46^{b}	25.73^{ab}	24.74^{bc}	24.62^{bc}	24.50^{c}	26.45^{a}	1.16	0.041
PUFA	39.74^{b}	40.47^{ab}	40.87^{ab}	40.43^{ab}	40.78^{ab}	41.24^{a}	1.44	0.045
Σ n-6/Σ n-3	9.01^{a}	8.03^{b}	7.41^{c}	8.32^{b}	8.25^{b}	7.12^{c}	0.29	0.031
AI	0.30	0.29	0.29	0.29	0.30	0.28	0.02	0.106
TI	0.75	0.71	0.70	0.72	0.72	0.64	0.04	0.053
h/H	3.62	3.72	3.67	3.64	3.63	3.88	0.12	0.097

$^{a, b, c}$ – values in the same rows with different letters differ significantly (p≤0.05)

fatty acid composition in heart (Table 6) have drawn attention to AI, TI and h/H indices that, despite the differences in fatty acid fraction shares (UFA, SFA, n-6/n-3), had rather similar values, irrespective of a dietary supplement included.

Discussion

Prebiotics, eubiotics and herbs are considered promising in-feed supplements used in swine nutrition. Through biologically active substances (fructooligosaccharides (FOS), flavonoids, organic acids, alkaloids, glycosides, etc.), they can affect animal metabolism directly or indirectly – stimulating the proliferation of beneficial microbiota, *Lactobacillus* or *Bifidobacterium*, in the gastrointestinal tract (Bornet et al. 1997, Beylot 2005). The present paper investigates the impact of inulin preparations obtained from the water and water-alcohol extraction from chicory roots and inulin-rich plants, i.e. Jerusalem artichoke tubers, roots of chicory and dandelion, on blood lipid profile and fatty acid composition in backfat, m. *longissimus dorsi*, heart and liver as well as atherogenicity and thrombogenicity indices and hypo/hypercholesterolemic acid ratio.

A level of the studied biochemical parameters of fatteners blood plasma was found within the reference range (Kuleta et al. 1993, Winnicka 2008). No significant differences in the analyzed lipid indices subject to a method of inulin extraction were determined. Slightly more favorable findings were obtained in group III during the first fattening period (decreased TCh and TG level) when water-alcohol extract was used which, besides inulin, also contained other sugar compounds and flavonoids. According to Cieślik et al. (2005) dietary inulin induced significant increases in serum HDL-cholesterol concentrations and significantly decreased the level of triacylglycerols. The addition of dried chicory root (60% FOS) and dandelion (50% FOS) has significantly lowered blood total cholesterol in fatteners. Sesquiterpene lactones, triterpenoids and sterols present in dandelion root (Yan et al. 2011) or chicory (Mila et al. 2009) are the substances that, besides inulin and other FOSs, are known to promote animal health, lower total cholesterol and triglyceride level, what have been confirmed in the present researches. *Taraxacum officinale* has also been used as a medicinal herb for both humans or animals for a long time because of its anti-inflammatory, anti-oxidative and anti-allergic activity (Cheong et al. 1998, Hagymasi et al. 2000).

Inulin extracts or inulin-storing plant material contributed to decreased cholesterol content in fattener tissues and the most favorable changes (statistically significant differences) were observed between the control and group VI (with dandelion root powder). Apart from FOSs, the compounds contained in the dandelion root (among others, flavonoids, triterpenoids and sterols) modify cholesterol metabolism primarily due to the reduction of *de novo* fatty acid synthesis in the liver, enhance its expulsion from the body and, thus, tend to reduce the cholesterol being deposited in tissues and organs (Rimm et al. 1996, Beylot 2005).

Fatty acid proportions or their fractions (PUFA, SFA, MUFA) can be modified through the use of animal nutritional strategies, including herb and oligosaccharide feed additives (Beylot 2005, Fernández et al. 2007). The inulin preparations used in the experiment were obtained by two different extraction methods and exhibited only a slightly different content of inulin, disaccharides and other compounds. Essentially, they did not show significant differences in fatty acid fractions or AI, TI and h/H ratio in the analyzed tissues and organs (Table 3-6). Studying other factors of variation, i.e. inulin-storing plant material in dried form of topinambur tubers, chicory and dandelion roots, far more noticeable effect on fatty acid composition as well as AI and TI indices was established in backfat (Table 3), *longissimus dorsi* muscle fat (Table 4), liver (Table 5) and heart (Table 6). The highest PUFA percentage and a beneficial ratio between n-6/n-3 acids were shown in the fat of *longissimus dorsi* muscle, liver and heart of fatteners from group VI fed a dandelion powder-supplemented diet. The favorable effects induced by a dietary additive of dried dandelion root result from a variety of biologically active substances it comprises that act upon lipid metabolism of organism (Yarnell and Abascal 2009, Yan et al. 2011).

Conclusion

The addition of 20 g inulin preparation per 1 kg mixture, irrespective of an extraction method, from chicory root, did not affect significantly cholesterol content in blood plasma or fat of the *longissimus dorsi* muscle, liver and heart of the fatteners. Alike, no significant changes were determined in the fatty acid profiles and AI, TI and h/H ratio in the analyzed pig tissues and organs. Dietary inclusion of 40 g of dried Jerusalem artichoke and chicory supplement per 1 kg mixture did not have an explicit effect on the analyzed indices of fatteners blood and tissues. Beneficial and statistically significant changes in blood lipid profile (the highest HDL-cholesterol content) and fatty acid composition (elevated PUFA level and declined n-6/n-3 ratio and AI, TI, h/H indices) resulted from the supplementation of 1 kg diet with 40 g of dandelion powder. Fatty acid composition and the cal-

culated AI, TI, h/H indices of backfat and meat as well as heart and liver of the fatteners fed a diet enriched with dried dandelion indicate the reduced risk for developing atherosclerotic disorders and, therefore, may be recommended for human nutrition.

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