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Effect of dietary inulin source on piglet performance, immunoglobulin concentration, and plasma lipid profile

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Abstract

The aim of the study was to evaluate the effect of an inulin source (aqueous or aqueous-alcoholic extract, dried chicory root, or dried Jerusalem artichoke tubers) on piglet performance, plasma lipid profile, and immunoglobulin concentration. The study was conducted on 534 piglets (44 litters) allocated to five nutritional groups: group I (control) – fed basal diet, groups II and III receiving basal diet supplemented with 2% of inulin (aqueous and aqueous-alcoholic extract respectively), and groups IV and V – 4% additive of dried artichoke or dried chicory respectively. During the trial, piglets' body weight, feed intake, diarrhoea incidence, and mortality were controlled. Blood samples were collected twice from six animals of each group. In blood plasma, indices of lipid profile and concentrations of IgA, IgG and IgM were measured. The addition of inulin, regardless of its form (extracts or dried plants), significantly improved the rearing indices. In piglets of groups III, IV and V a significant improvement of daily weight gains and feed efficiency was noted. Inulin showed hypolipidemic activity (lowered total cholesterol level) and stimulated piglet immune system manifested by elevated IgA and IgG concentrations. Irrespective of the inulin source, a lower mortality rate resulting from the improvement of animal health was noted in all experimental groups.

Keywords: piglet, inulin, chicory, Jerusalem artichoke, animal performance, immunoglobulins, lipids.

Introduction

Inulin is one of fructans naturally occurring in many plants, mostly extracted from chicory root (*Cichorium intybus*) or Jerusalem artichoke (*Helianthus tuberosus*). It is composed of oligo- and polysaccharides, which give inulin its unique prebiotic properties (19). Notably, thanks to β-(2-1) glycosidic bonds, inulin is resistant to digestive enzymes of the small intestine in monogastric animals. Through the bacterial fermentation process, inulin stimulates the growth of health-promoting bacteria and at the same time, suppresses potentially pathogenic microorganism growth in the distal portion of the gastrointestinal tract (27). The microflora produces a number of nutraceutical compounds like organic acids, hydrogen peroxide, acidolin, acidolphilin, reuterin, lysozyme, lactoferrin, lactoperoxidase, as well as bacteriocins such as lactocidine or lactocin, which exhibit immunomodulatory and antimicrobial potential (4, 5). Inulin promotes the growth of lactic acid bacteria and thus exerts an indirect beneficial effect on the host immune system through, among others, production of cytokines, mononuclear cells, and phagocytising macrophages. Besides, inulin contributes to the induction of immunoglobulin synthesis, especially IgA (16, 26). Inulin fructooligosaccharides induce proliferation of beneficial microflora (*Bifiobacterium* and *Lactobacteriaceae*), which as probiotic bacteria improve animal rearing performance (1, 4). It is pivotal at the post-weaning period characterized with high incidence of diarrhoea leading to death of piglets. Prevention of post-weaning digestive disorders, including feed additive introduction, can increase rearing efficiency.

The objective of the research was to assess the effect of inulin additive given as aqueous or aqueous-alcoholic extract, or dried artichoke or chicory on piglet production efficiency and some blood biochemical parameters.

Material and Methods

Animals and study protocol. All experimental procedures were approved by the Local Ethical Committee of the Faculty of Biology and Animal Breeding at the University of Life Sciences in Lublin.

Five hundred and thirty four piglets (94, 96, 100, 120 and 124 in groups I, II, III, IV and V respectively) of PIC hybrid line sows mated by Penarlan P76 boars were used in the trial. Depending of the inulin source, the piglets were assigned into five groups: control group (I) fed basal diet and four experimental groups supplemented with 20 g of aqueous (group II) or aqueous-alcoholic (group III) extracts of inulin as well as dried artichoke tubers (group IV) or dried chicory root (group V) in amount of 40 g kg^{-1} of complete feed. Inulin was extracted from chicory root according to the modified method of Stahl and Schild (21) and the substrate was treated with water only (aqueous extract) or in 70:30 water to ethyl alcohol ratio solution (aqueous-alcoholic extract). The experimental diets (Table 1) were introduced upon the onset of supplementary feeding of animals (about 10 – 12 d of life) until 84 d. The feeds and water were provided *ad libitum*. Animal body weight, feed intake, diarrhoea incidence and mortality rate were controlled on days 10, 28, 49, and 84 of piglets' life.

Blood samples. Blood samples were collected from six animals of each group twice: at weaning (28 d) and after the rearing period completion (84 d). In blood plasma, the content of total cholesterol (CHOL), triacylglycerols (TG), and HDL cholesterol fraction (HDL-Chol) was measured by colorimetric methods using Biomaxima monotests. The low density lipoprotein (LDL-Chol) was calculated using Friedewald (7) formula:

LDL-Chol (mmol L^{-1}) = Total cholesterol – HDL-Chol – triacylglycerols /2.2.

The level of IgG, IgA, and IgM was also measured using the ELISA method (Bethyl Laboratories Inc.). The obtained numerical data were analysed with analysis of variance (ANOVA), while significance of differences between the average values of the analysed traits was determined with Tukey's test for unequal sample sizes.

Table 1. Composition (g kg^{-1}) of piglets' diets

Components	Experimental groups				
	I	II	III	IV	V
Wheat 11.5% TP	478.41	478.41	478.41	438.41	438.41
Barley 10.5% TP	200	200	200	200	200
Corn starch	20	-	-	20	20
Inulin (aqueous extract)	-	20	-	-	-
Inulin (aqueous-alcoholic extract)	-	-	20	-	-
Dried Jerusalem artichoke	-	-	-	40	-
Dried chicory	-	-	-	-	40
Full fat soybean extruded	59	59	59	59	59
Dried sweet whey 70% TP	97	97	97	97	97
Fish meal 70% TP	40	40	40	40	40
Blood plasma 80% TP	40	40	40	40	40
Soybean oil	33.9	33.9	33.9	33.9	33.9
Calcium formate	3	3	3	3	3
Chalk	5	5	5	5	5
Calcium monophosphate	6	6	6	6	6
Salt	0.7	0.7	0.7	0.7	0.7
L-lysine HCl 78.5%	6.14	6.14	6.14	6.14	6.14
Dl-methionine 99%	2.32	2.32	2.32	2.32	2.32
L-threonine 98%	2.64	2.64	2.64	2.64	2.64
L-tryptophan 98%	0.89	0.89	0.89	0.89	0.89
Premix prestarter*	4	4	4	4	4
Flavour	1	1	1	1	1

*1 kg of premix contained: vitamin A 600 000 i.u., D_3 60 000 i.u., E 3000 mg, K_3 120 mg, B_1 120 mg, B_2 240 mg, B_6 240 mg, nicotinic acid 1600 mg, pantothenic acid 800 mg, folic acid 160 mg, biotin 10 mg, B_{12} 1.6 mg, chloride choline 12 g, Mg 0.8 g, Fe 6 g, Zn 5.6 g, Mn 2.4 g, Cu 6.4 g, J 40 mg, Se 16 mg, Co 16 mg

Results

Regardless of the inulin source, animal survival rate in all experimental groups was higher than that in the control group ($P \leq 0.05$). The number of piglets in litters, from the gilts assigned to each group, ranged from 11.8 (group I) up to 12.5 (group III) (Table 2). The lowest pre-weaning death losses were noted in group II receiving inulin aqueous extract, while interestingly, the post-weaning mortality rate of piglets was the highest in this group and control group. Throughout the entire observation period (till 84 d of life), the lowest death rates were recorded in groups II and III where diets were supplemented with inulin extracts, whereas the greatest in group I ($P \leq 0.05$). Dietary inclusion of inulin during the rearing period reduced piglet mortality by even 11 percentage points (group III). Piglet deaths were primarily caused by overlaying (crushing), low viability (low body weight at birth), and diarrhoea. Significant differences were also noted in frequency and severity of diarrhoea. The groups receiving a mixture comprising inulin additive had lower death loss rate related to digestive disorders and, importantly, diarrhoea duration periods were shorter (2–3 d) as compared to the control group (4–5 d).

The highest weight gains during 10 – 84 d of life were observed in piglets receiving an additive of Jerusalem artichoke (group IV) or chicory root (group V), as well as aqueous-alcoholic extracted inulin (group III). Dietary inclusion of powdered ingredient, especially chicory root, has enhanced animal appetite. Although no statistically significant differences in feed conversion throughout the study period were noted, the effect of the introduced feed supplements was noticeable during the critical pre-weaning time (10 – 28 d of life) and late rearing period (50 – 84 d of life). The lowest feed consumption was recorded in groups III and IV, where the animals were fed diet comprising aqueous-alcoholic extract of inulin or dried artichoke (Table 2).

In the late rearing period (84 d of life), a decrease in total cholesterol level ($P \leq 0.05$) was noted in blood plasma of the animals supplemented with dried plants (group IV and V) and aqueous-alcoholic extract of inulin (group III) (Table 3). In the all experimental groups, a lower LDL cholesterol content was observed as compared to the control group, which also contributed to higher share of HDL fraction in total cholesterol (Table 3).

The investigated feed additives did not have significant impact on concentration of immunoglobulin M (Table 4). Though, IgA concentration tended to elevate on the weaning day, whereas significant differences on 84 rearing day in groups II and III were observed as compared with the control. Besides, significant differences were also reported in IgG concentration. The lowest IgG content was recorded in the control group at weaning and final period of experiment ($P \leq 0.05$).

Table 2. Rearing results

Item	Control	Inulin source				*P*
		Extracts		Dried plants		
Groups	I	II	III	IV	V	
Number of litters	8	8	8	10	10	
Number of piglets per litter:						
at birth	11.8	12.0	12.5	12.0	12.4	0.083
at 10 d	11.3	11.5	12.3	11.6	12.0	0.058
at 28 d	10.8	11.5	11.5	10.6	11.2	0.073
at 49 d	9.8^{a}	10.8^{ab}	11.5^{b}	10.4^{ab}	11.0^{ab}	0.047
at 84 d	9.5^{a}	10.8^{ab}	11.5^{b}	10.4^{ab}	10.8^{ab}	0.044
Losses of piglets, %:						
up to 10 d of age	4.25^{b}	4.17^{b}	2.00^{a}	3.33^{ab}	3.23^{ab}	0.014
up to 28 d of age	8.51^{b}	4.17^{a}	8.00^{b}	11.67^{c}	9.68^{bc}	0.007
up to 49 d of age	17.02^{c}	10.42^{ab}	8.00^{a}	13.33^{b}	11.29^{ab}	0.013
up to 84 d of age	19.15^{c}	10.42^{ab}	8.00^{a}	13.33^{b}	12.90^{b}	0.005
due to diarrhoea, %	5.48^{a}	3.70^{b}	1.23^{c}	3.67^{b}	2.04^{bc}	0.035
ADG, g:						
10 – 28 d	271.5^{ab}	264.8^{b}	281.8^{a}	283.4^{a}	292.1^{a}	0.042
29 – 49 d	341.1^{a}	334.0^{a}	283.0^{b}	294.7^{b}	277.8^{b}	0.032
50 – 84 d	360.8^{b}	373.2^{b}	440.4^{a}	450.6^{a}	434.8^{a}	0.029
10 – 84 d	333.5^{b}	335.7^{b}	357.1^{a}	365.7^{a}	355.5^{a}	0.038
Feed intake, g d^{-1}:						
10 – 28 d	73.1^{a}	65.3^{b}	63.8^{b}	54.7^{c}	59.6^{bc}	0.033
29 – 49 d	604.4^{a}	543.3^{b}	495.5^{c}	486.0^{c}	487.2^{c}	0.029
50 – 84 d	1064.3^{b}	1093.3^{ab}	1105.3^{ab}	1180.0^{a}	1198.6^{a}	0.045
10 – 84 d	692.7^{ab}	687.1^{ab}	678.9^{b}	709.3^{ab}	719.7^{a}	0.046
FCR, kg kg^{-1}:						
10 – 28 d	0.27^{a}	0.28^{a}	0.23^{b}	0.20^{b}	0.20^{b}	0.034
29 – 49 d	1.80	1.67	1.79	1.66	1.76	0.058
50 – 84 d	2.97^{a}	2.95^{a}	2.54^{b}	2.64^{b}	2.76^{ab}	0.032
10 – 84 d	2.08	2.05	1.91	1.94	2.02	0.058

Groups: I – control, II – aqueous extract, III – aqueous-alcoholic extract, IV – dried Jerusalem artichoke, V – dried chicory
$^{a, b, c}$- values in rows with different letters differs significantly ($P \leq 0.05$)

Table 3. Plasma lipid profile

Parameter	Day of life	Control	Inulin source				*P*
			Extracts		Dried plants		
		I	II	III	IV	V	
Total cholesterol, mmol L^{-1}	28.	4.16	4.05	4.20	4.05	4.03	0.123
	84.	4.73^{a}	4.61^{a}	4.25^{b}	4.27^{b}	4.08^{c}	0.042
Triacylglycerols, mmol L^{-1}	28.	0.71	0.59	0.64	0.53	0.63	0.061
	84.	0.59	0.52	0.49	0.47	0.54	0.058
HDL-Chol, mmol L^{-1}	28.	1.76	1.78	1.65	1.72	1.81	0.094
	84.	2.01	2.09	1.94	1.97	2.08	0.054
LDL-Chol, mmol L^{-1}	28.	2.08^{ab}	2.00^{ab}	2.25^{a}	2.09^{ab}	1.94^{b}	0.045
	84.	2.36^{a}	2.28^{a}	2.09^{ab}	2.08^{ab}	1.75^{b}	0.042
Cholesterol/HDL	28.	2.36^{ab}	2.27^{b}	2.54^{a}	2.35^{ab}	2.23^{b}	0.041
	84.	2.25^{a}	2.20^{ab}	2.19^{ab}	2.16^{ab}	1.96^{b}	0.049
% HDL	28.	42.33^{ab}	44.02^{a}	39.35^{b}	42.52^{ab}	44.78^{a}	0.037
	84.	44.36^{b}	45.43^{b}	45.61^{b}	46.26^{b}	51.07^{a}	0.021

$^{a, b}$ - values in rows with different letters differs significantly ($P \leq 0.05$)

Table 4. Immunoglobulin concentration in piglets' blood plasma

Parameter	Day of life	Control	Inulin source				*P*
			Extracts		Dried plants		
		I	II	III	IV	V	
IgA, mg mL^{-1}	28.	0.29	0.34	0.35	0.31	0.32	0.052
	84.	0.73^{b}	0.85^{a}	0.86^{a}	0.78^{ab}	0.79^{ab}	0.047
IgG, mg mL^{-1}	28.	6.40^{b}	7.52^{a}	7.65^{a}	6.79^{b}	7.31^{a}	0.043
	84.	6.72^{b}	7.63^{a}	7.68^{a}	7.19^{ab}	7.27^{ab}	0.034
IgM, mg mL^{-1}	28.	0.78	0.80	0.82	0.74	0.75	0.104
	84.	1.99	1.94	1.98	2.07	2.04	0.093

$^{a, b}$ - values in rows with different letters differs significantly ($P \leq 0.05$)

Discussion

The present research results showed that inulin addition has positively affected overall animal productivity. Lower death loss rate related to digestive disorders and shorter diarrhoea duration periods in groups receiving inulin, irrespective of its source, as compared to the control group, indicate the stabilising effect of inulin, as a prebiotic, on piglets intestinal microflora population and digestive tract functioning, thus consequently improves production efficiency. The piglets fed a diet with inulin from aqueous-alcoholic extraction (group III) and dried Jerusalem artichoke (group IV), or chicory root (group V) showed substantially higher daily body weight gains ($P \leq 0.05$) throughout the rearing period until 84 d of life. Interestingly, immediately after weaning, lower weight gains were recorded in the same groups comparing to the control. It corresponds with a considerable increase in mortality rate and decreased feed intake observed in these groups. However, this period was greatly compensated by the faster growth in the late fattening, which finally improved overall rearing efficiency. Similarly, He *et al.* (10), in their study on 180 piglets proved that feeding diets supplemented with inulin (added to feed or water) increased daily weight gains of the animals. Alike, Kjos *et al.* (13) highlighted a significant increase in daily weight gains at the early and late fattening period with increasing inulin content in diet. Abe *et al*. (1) also reported improved performance parameters in piglet rearing (low diarrhoea incidence and mortality rate) when diets were supplemented with *Bifidobacterium pseudolongum* or *Lactobacillus acidophilus*, which growth was stimulated by prebiotics, including inulin. Whereas, other studies conducted on piglets showed slightly better weight gains recorded only in the first 14 study days (6). Summart *et al.* (22) incorporated Jerusalem artichoke additive into 21-day-old piglet diet for five weeks and obtained higher ADGs but the differences were not statistically significant.

A significantly better feed conversion rates, especially at the time when piglets stayed with sows (10–28 d) and at late rearing period (50–84 d), were noted in groups III, IV, and V with dietary inclusion of inulin from aqueous-alcoholic extraction, dried artichoke, or chicory root, respectively. Similarly, the studies of other authors (6, 11, 13) demonstrated more efficient feed conversion after inulin introduction into animal feed.

Numerous studies on human and animals showed dietary fructans to have hypolipidemic properties manifested by lowered plasma total cholesterol and triacylglycerol content (2, 14, 23, 24). These properties may result from a declined absorption of nutrients, enhanced excretion of bile acids, or boosted propionic acid production by gastrointestinal tract

microorganisms (3). Biochemical values obtained in the present study, were mostly within the reference range (8, 25). A downward trend in total cholesterol and triacylglycerol content in blood plasma of animals fed diet supplemented with inulin along with noticeable higher share of HDL cholesterol fraction as compared with the control group was noted (Table 3). Considering the cholesterol/HDL and percentage of HDL parameters, the animals receiving a mixture with dried chicory root additive (group V) had the most desirable parameters of blood lipid profile. Kim and Shin (12), in their research on rats fed chicory root extract (1% and 5%) or inulin (5%) as dietary supplement, demonstrated beneficial effect of the additives on blood lipid profile. Liong *et al.* (15) also observed lower total cholesterol content following the implementation of synbiotic comprising inulin and probiotic bacteria into pig diets.

Promotion of beneficial bacteria growth and inhibitory activity against pathogens indicate immunostimulatory properties of prebiotics, including inulin (17, 20). Prebiotics may have direct or indirect effect on some immune mechanisms in animals and thus improve animal health and performance. They can directly improve health status through stimulation of the activity of phagocytic cells as well as nonspecific humoral immunity mechanisms. The studies of other authors (9, 16, 18, 26) showed that prebiotic supplementation, including inulin, enhances synthesis and secretory activity of IgA in the gastrointestinal tract. The present research included the analysis of the concentration of immunoglobulins class A, G, and M in piglet blood plasma. The elevated IgA concentration in the late rearing period ($P < 0.05$) was found in groups II and III receiving inulin extract, irrespective of the method of its extraction. In all groups with dietary inulin supplementation, a higher concentration of IgG was noted as compared to control group; however, the highest IgG level was observed in groups with inulin extract additive (groups II and III) as compared to those fed dried products (groups IV and V).

Summing up, inulin as feed supplement, regardless of its source (extracts or dried inulin-generating plants), has clearly improved animal rearing indices. The piglets fed a diet containing inulin from aqueous-alcoholic extraction as well as dried Jerusalem artichoke or chicory showed substantially improved daily weight gains and better feed conversion. Irrespective of the inulin source, piglets' survival rate was higher in all experimental groups. Inulin shows hypolipidemic activity (lowered total cholesterol level) and stimulates piglet systemic immune system as it was manifested by elevated IgA and IgG concentrations.

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