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MICROBIAL ACTIVITY IN THE LARGE INTESTINE OF CHICKS FED DIETS WITH DIFFERENT TYPES AND LEVELS OF INULIN*

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Abstract

The aim of the study was to evaluate the effects of dietary level of two types of inulin differing in the degree of polymerization (DP) on microbial activity in the large intestine of chicks. The experiment was performed on 70 one-day-old Ross 308 male chicks divided into 7 groups fed starter-type diets without inulin addition or supplemented with 0.2%, 0.4% or 0.6% of inulin with DP≥10 (IN_{10}), or DP≥23 (IN_{23}). After 14 days of feeding birds were sacrificed to collect digesta samples from caeca and colon. Caecal digesta was examined for pH, concentrations of short-chain fatty acids (SCFA) and amines, activities of β-glucosidase and mucinase, and relative amount of selected bacterial populations, whereas in colonic digesta only pH, SCFA and amines were analysed. Regardless of DP, inulin level did not affect digesta pH, activity of bacterial enzymes and relative amounts of *Clostridium* spp., *Lactobacillus* spp., *Bifidobacterium* spp. and *E. coli* populations. Dietary level of IN_{10} significantly affected propionic acid concentration, which was greater in caecal digesta of birds fed diet supplemented with 0.2% of IN_{10} compared to other groups and feeding diets supplemented with all levels of IN_{10} significantly reduced histamine concentration compared to the control. There were no effects of inulin on microbial activity indices in the colon. The present study indicates that, regardless of DP, inulin does not modify considerably microbial activity in the large intestine of chicks.

Key words: inulin, chicks, SCFA, amines, bacterial populations

The bacterial community of the gastrointestinal tract has a major impact on its functions, and thereby can also influence animal health and productivity. Large numbers of bacteria species and the end-products of fermentation are all likely to have effects on animal health. One of the most important are short-chain fatty acids (SCFA). These compounds are bacteriostatic or bactericidal *in vitro* for Gram negative bacteria, provided that they are sufficiently dissociated and are in contact with

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the bacteria cells for a sufficiently long time. Furthermore, production of SCFA by caecal microflora of broiler chickens is responsible for preventing colonization of the gastrointestinal tract by pathogens, including Salmonella (Thompson and Hinton, 1997; Corrier et al., 1995).

Biogenic amines, particularly polyamines, are involved at almost every step of the synthesis of nucleic acids and protein and are necessary for many processes such as cell growth, renewal and metabolism (Önal et al., 2013). However, amines originate from protein degradation and high bacterial production of these putrefactive compounds in the gut can induce several toxicological problems (Windey et al., 2012).

However, intestinal bacteria metabolise also exo- and endogenous substances, and increased activity of some enzymes, e.g. mucinase and β-glucosidase is a risk factor of pathogenesis. Mucinase is used by pathogens to degrade protective mucus layer which facilitates invasion (Almagro-Moreno et al., 2015; Valeri et al., 2015), whereas β-glucosidase is a hydrolase activating toxins and carcinogens in the large intestine (Desrouillères et al., 2015).

Nutrients modulating microbiota composition and activity, such as constituents of dietary fibre, affect the intestinal environment and have been used as prebiotics to promote chicken health (Apajalahti et al., 2004; Ganguly, 2013; Sugiharto, 2014). Inulin is a non-digestible carbohydrate present in many vegetables, fruits and cereals. The source of fructooligosaccharides (FOS) and inulin, by far most widely exploited by the industry, is a root of chicory (*Cichorium intybus* L.). Inulin consists of one terminal α-glucose molecule and a variable number of β-fructose moieties exclusively linked by β (2 → 1) glycosidic bonds (Vergauwen et al., 2003). Degree of polymerization (DP) typically ranges from 2 to 60 units, with an average of 10–12 units (Madrigal and Sangronis, 2007). Fructans with DP lower than 10 are known as oligofructose or FOS. Owing to their chemical structure, inulin-type fructans resist the breakdown by digestive enzymes in the small intestine of animals but are degraded by microbiota, mainly in the large intestine. Inulin-type fructans are assumed to modulate the composition of microbiota by stimulation of growth and activity of certain beneficial bacteria and inhibition of growth of potential pathogens in the gastrointestinal tract of chickens.

Oligosaccharides have been shown to influence the intestinal bacterial community (Cao et al., 2005; Rehman et al., 2008) in broilers, but there are also some reports indicating that this supplement does not affect the intestinal bacteria (Biggs et al., 2007). Dietary level of inulin, DP and developmental stage of the gut have to be considered and may explain varying effects on the microbiota activity indices. In the first days of broiler chicks' life bacterial counts and acetate, propionate and butyrate levels are quite low in the caecum, then increase and reach a plateau by approximately 15 days of age (van der Wielen et al., 2000). Before the plateau, prebiotic feed additives may be used to improve microbial activity in the large intestine, ensuring optimal health status and growth performance of broilers. The study was therefore performed to evaluate the effect of dietary level of two types of inulin, differing in DP, on microbial activity in the large intestine of broiler chickens in the early post hatch period.

Material and methods

The design of the study and experimental procedures were approved by the Third Local Animal Experimentation Ethics Committee (Resolution no. 30/2010, Warsaw University of Life Sciences-SGGW, Warsaw, Poland) according to the principles of European Union and the Polish Law on Animal Protection. A total of 70 one-day-old Ross 308 male broiler chicks, obtained from a commercial hatchery, were weighed and allocated to seven groups of 10 birds each, so that the mean initial body weight was equal. Birds were fed starter-type diet without inulin addition (control group, C) or with inulin from chicory root with DP ≥ 10 (IN_{10}) or DP ≥ 23 (IN_{23}) (Inulin Orafti®GR or Inulin Orafti®HPX, respectively; Orafti Beneo GmbH, Mannheim, Germany) at 0.2%, 0.4% or 0.6% level. Isoprotein and isoenergetic diets (Table 1) were formulated according to the Polish recommendations for broilers (Smulikowska and Rutkowski, 2005) and produced by the same feed mill in Morawski Feed Production Plant (Kcynia, Poland). Birds were kept in an electrically heated battery brooders on wire-bottom floor (five birds per cage, two cages per group) with free access to feed and water, in a room with temperature of 30°C during the first three days and thereafter gradually reduced, and light cycle consisting of 18 h light and 6 h darkness. Feed intake was measured weekly. After 14 days of feeding birds were weighed and sacrificed by decapitation. The abdominal cavity was opened and caeca and colon were excised for sample collection. Caecal digesta was collected for analyses of pH, SCFA, amines, bacterial enzyme activity and bacterial populations, whereas colonic digesta was taken for pH, SCFA and amines analyses.

Analyses

The chemical composition of the experimental diets was analysed according to standard procedures of the Association of Official Analytical Chemists (AOAC) (2000).

The caecal and colonic digesta samples were mixed immediately with deionized water and pH was measured by a digital pH-meter (WTW GmbH, Weilheim, Germany.) The SCFA analysis was performed according to the procedure described by Barszcz et al. (2011) using an isocaproic acid as an internal standard and HP 5890 Series II gas chromatograph (Hewlett-Packard, Waldbronn, Germany) equipped with a flame-ionization detector and Supelco Nukol fused silica capillary column (30 m × 0.25 mm i.d.; 0.25 μm). Helium was used as the carrier gas. The initial column temperature was set at 100°C for 2 min, then heated at 10°C/min to 140°C and held at the final temperature for 20 min. The injector temperature was maintained at 220°C, while detector was kept at 250°C. Concentrations of individual SCFA were estimated in relation to the internal standard using a mixture of SCFA standard solutions.

Concentration of amines in caecal and colonic digesta of birds were determined according to the method of Taciak et al. (2015). The digesta sample was homogenised in ultra-pure water by intensive vortexing and the supernatant was obtained by centrifugation at 10 000 rpm for 15 min. After that, amines were derivatized with dansyl chloride in acetone and extracted using Waters SEP-PAK serif™ C18 cartridges. Before extraction heptylamine was added as the internal standard. Finnigan Sur-

veyor Plus HPLC (Thermo Scientific, San Jose, USA) equipped with photodiode array detector, pre-column (Waters Symmetry Shield RP_{18}, 20 × 3.9 mm, 5.5 µm) and column (Waters Symmetry Shield RP_{18}, 150 × 3.9 mm, 5.5 µm) was used for this analysis. Mobile phase was composed of acetonitrile/water (5/95%, v/v) mixture and acetonitrile (100%), flowing under a gradient elution with 0.7 ml/min.

Table 1. Ingredient and chemical composition of the experimental diets (g kg^{-1})

Item	C	IN_{10}			IN_{23}		
	0%	0.2%	0.4%	0.6%	0.2%	0.4%	0.6%
Ingredients (g kg^{-1})*							
wheat	203.2	203.2	203.2	203.2	203.2	203.2	203.2
corn	390	390	390	390	390	390	390
corn starch	10	8	6	4	8	6	4
inulin IN_{10}	-	2	4	6	-	-	-
inulin IN_{23}	-	-	-	-	2	4	6
soybean meal	332.9	332.9	332.9	332.9	332.9	332.9	332.9
monocalcium phosphate	13	13	13	13	13	13	13
soybean oil	24	24	24	24	24	24	24
limestone	7.6	7.6	7.6	7.6	7.6	7.6	7.6
$NaHCO_3$	1.2	1.2	1.2	1.2	1.2	1.2	1.2
NaCl	2.7	2.7	2.7	2.7	2.7	2.7	2.7
lysine HCl	2.1	2.1	2.1	2.1	2.1	2.1	2.1
DL-methionine	2.8	2.8	2.8	2.8	2.8	2.8	2.8
L-threonine	0.5	0.5	0.5	0.5	0.5	0.5	0.5
mineral and vitamin premix**	10	10	10	10	10	10	10
Chemical composition							
DM	894.3	894.9	895.4	894.3	894.8	895.6	894.8
crude ash	55.9	55.8	55.8	55.7	55.8	55.8	55.7
crude protein	215.5	215.3	215.2	215.1	215.4	215.2	215.1
ether extract	61.4	61.4	61.3	61.3	61.4	61.2	61.2
crude fiber	25.4	25.2	25.2	25.1	25.2	25.2	25.1
MEN (MJ/kg^{-1})***	12.6	12.6	12.6	12.6	12.6	12.6	12.6

*All diets were supplemented with anticoccidial agent Cycostat 66G (0.5 g/kg diet).

** Provided per kilogram of diet: Fe (from ferrous sulphate), 70 mg; Cu (from copper sulphate), 8 mg; Zn (from zinc oxide), 60 mg; Mn (from manganese sulphate), 70 mg; I (from calcium iodate), 1 mg; Se (from sodium selenite), 0.3 mg; retinyl acetate, 3.06 mg; cholecalciferol, 0.0625 mg; α-tocopherol acetate, 25 mg; menadione, 3 mg; thiamin, 2 mg; riboflavin, 6 mg; pantothenic acid 15 mg; choline chloride, 700 mg; nicotinic acid 30 mg; pyridoxine, 5 mg; biotin, 0.02 mg; folic acid, 2 mg; vitamin B_{12} (cobalamin), 0.02 mg.

*** MEN was calculated based on chemical analyses of feeds and tabular data (Smulikowska and Rutkowski, 2005; Sauvant et al., 2004).

For analyses of bacterial enzymes activity (β-glucosidase and mucinase) caecal digesta samples (ca. 0.5 g) were mixed with 5 ml of ice-cold phosphate buffered saline (PBS), pH 7.4, homogenized for 30 s, sonicated (2×30 s) and centrifuged (10 000 rpm, 20 min, room temperature). Supernatants were stored at -80°C until analyses. Activity of β-glucosidase was determined according to a modified method of Juśkiewicz et al. (2004). Substrate solution (5 mM *p*-nitrophenyl-β-D-

glucopyranoside) was added in the amount of 150 µl to a disposable polystyrene cuvettes and, after 5 min of preincubation at 37°C, was mixed with 100 µl of the supernatant. After 10 min of incubation at 37°C, enzymatic reaction was terminated by the addition of 1250 µl of an ice-cold, 0.25 M sodium carbonate and the absorbance was measured at 400 nm using a Unicam UV300 spectrophotometer (Thermo-Spectronic, Cambridge, UK). The activity of β-glucosidase was calculated from a standard curve for *p*-nitrophenol.

Table 2. Primers for PCR amplification of bacterial 16S rRNA

Bacterial group	Primers	Sequence (5'-3')	Product length (base pairs)	Reference
Universal	Forward	CGTGCCAGCCGCGGTAATACG	611	Amit-Romach et al., 2004
	Reverse	GGGTTGCGCTCGTTGCGGGACT-TAACCCAACAT		
Lactobacillus spp.	Forward	CATCCAGTGCAAACCTAAGAG	286	Wang et al., 1996
	Reverse	GATCCGCTTGCCTTCGCA		
Escherichia coli	Forward	GGGAGTAAAGTTAATACCTTTGCTC	585	Tsen et al., 1998
	Reverse	TTCCCGAAGGCACATTCT		
Clostridium spp.	Forward	AAAGGAAGATTAATACCGCATAA	722	Amit-Romach et al., 2004
	Reverse	ATCTTGCGACCGTACTCCCC		
Bifidobacterium spp.	Forward	CGGGTGCTICCCACTTTCATG	1417	Kaufmann et al., 1997
	Reverse	GATTCTGGCTCAGGATGAACG		

Mucinase activity was analysed based on the amount of reducing sugars as described by Shiau and Chang (1983) with the following modifications. Supernatant was diluted twice with PBS, pH 7.4, and placed (225 µl) into eppendorf tube. After 2 min of preincubation at 30°C, 25 µl of 0.5% porcine gastric mucin solution was added and incubated for 25 min. The reaction was stopped by heating the tubes at 100°C for 3 min. The amount of reducing sugars, released from mucin during incubation, was analysed spectrophotometrically at 540 nm and calculated from a standard curve for glucose.

Caecal digesta samples for isolation of bacterial genomic DNA was prepared as described by Zhu et al. (2002). DNA was then extracted using Wizard® Genomic DNA purification kit (Promega, Madison, WI, USA) according to manufacturer's instruction. DNA concentration and purity were analysed using NanoDrop ND-1000 spectrophotometer (Thermo-Scientific, Wilmington, DE, USA). The primers used for PCR amplification of bacterial 16S rRNA are shown in Table 2. The universal primer set was used for determination of the total microbiota population. For PCR amplification, 5 µl of isolated DNA was added to 45 µl of PCR mixture containing: 25 µl of 2x PCR *TaqNova*-RED Master Mix (BLIRT S.A. DNA-Gdansk Division,

Gdansk, Poland), 5 µl of each primer and 10 µl of nuclease-free water. The PCR thermal conditions were as follows: for *Lactobacillus* spp. and *Escherichia coli* strain, one cycle of 94°C for 3 min, 30 cycles of 94°C for 30 s, 60°C for 1 min and 72°C for 50 s, and one cycle of 72°C for 15 min; for total bacteria, one cycle of 94°C for 3 min, 29 cycles of 94°C for 30 s, 60°C for 1 min and 72°C for 50 s, and one cycle of 72°C for 15 min; for *Clostridium* spp., one cycle of 94°C for 3 min, 35 cycles of 94°C for 30 s, 60°C for 1 min, 68°C for 2 min and one cycle of 68°C for 7 min (Tako et al., 2008); for *Bifidobacterium* spp. one cycle of 95°C for 5 min, 40 cycles of 95°C for 15 s, 60°C for 30 s, 72°C for 2 min and one cycle of 72°C for 7 min (Kastner et al., 2006). PCR products were separated by electrophoresis on 2% agarose gel, stained with ethidium bromide and quantified using ImageJ 1.47v program for densitometry measurements (National Institute of Mental Health, Bethesda, MD, USA). The density of each band of examined bacterial DNA was expressed in relation to the density of the universal primers product.

Statistical analyses

The main effects of dietary treatments were determined by one-way ANOVA performed separately for the control and IN_{10} groups and for the control and IN_{23} groups. Differences between treatments were analysed by the Tukey HSD test using STATGRAPHICS® Centurion XVI ver. 16.1.03 software (StatPoint Technologies, Inc., Warrenton, VA, USA). The effects were considered to be significant at $P \leq 0.05$ and trend was set up at $P \leq 0.1$.

Results

Digesta pH and concentrations of SCFA in caecal and colonic digesta are shown in Table 3. There was no effect of inulin on pH, regardless of DP, but it slightly affected SCFA concentrations in caecal digesta. Supplementation of diet with 0.2% of IN_{10} significantly increased concentration of propionic acid ($P \leq 0.05$), compared to other experimental groups. Concentration of butyric acid showed increasing tendency ($P \leq 0.1$) due to the addition of IN_{23} compared to the control group. Feeding diets supplemented with inulin did not affect SCFA concentrations in colonic digesta of chickens.

Feeding diets supplemented with inulin did not affect the concentrations of putrescine, cadaverine, tyrosine and spermidine in the caecal digesta (Table 4). The concentration of histamine ($P \leq 0.05$) was significantly lower in birds fed 0.2%, 0,4% and 0.6% IN_{10} diets than in the control group. Methylamine concentration presented decreasing tendency ($P \leq 0.1$) due to the addition of 0.6% of IN_{10} to the diet. The only amine detected in colonic digesta was putrescine, the concentration of which did not differ significantly between experimental groups. There was no effect of IN_{23} level on concentrations of amines in caecal and colonic digesta of birds.

The activity of β-glucosidase and mucinase in caecal digesta of birds was not affected by either IN_{10} or IN_{23} level (Table 5).

Table 3. Digesta pH and concentration of short-chain fatty acids (µmol g^{-1}) in the caecum and colon of chicks

	C	IN_{10}			IN_{23}			SEM		P	
	0%	0.2%	0.4%	0.6%	0.2%	0.4%	0.6%	IN_{10}	IN_{23}	IN_{10}	IN_{23}
Caecum											
pH	5.39	5.91	5.65	5.56	5.35	5.8	5.61	0.161	0.162	0.161	0.233
acetic acid	24.16	21.50	21.52	24.53	19.87	25.53	26.78	1.032	1.251	0.626	0.228
propionic acid	0.82 a	1.09 b	0.51 a	0.58 a	0.61	0.75	0.87	0.07	0.05	0.020	0.280
isobutyric acid	0.30	0.34	0.29	0.25	0.26	0.22	0.26	0.02	0.02	0.530	0.580
butyric acid	5.63	4.62	5.24	7.35	6.94	9.07	7.16	0.50	0.46	0.280	0.080
valeric acid	0.17	0.20	0.21	0.14	0.14	0.19	0.15	0.02	0.01	0.570	0.380
Colon											
pH	7.37	7.31	7.36	7.58	7.38	7.31	7.36	0.159	0.135	0.644	0.983
acetic acid	8.78	8.36	11.11	14.71	11.64	8.55	9.72	4.588	1.904	0.724	0.599
butyric acid	0.25	0.23	0.33	0.45	0.29	0.25	0.25	0.142	0.050	0.653	0.901
oropionic acid	0.18	0.18	0.22	0.42	0.22	0.19	0.16	0.109	0.030	0.335	0.502
valeric acid	0.97	0.91	1.61	0.95	1.45	0.09	1.29	0.454	0.520	0.768	0.336

a, b – means in the same row with a different letters differ significantly at $P \leq 0.05$.

Table 4. Concentration of amines in the caecal and colonic digesta of chicks (nmol g^{-1})

	C	IN_{10}			IN_{23}			SEM		P	
	0%	0.2%	0.4%	0.6%	0.2%	0.4%	0.6%	IN_{10}	IN_{23}	IN_{10}	IN_{23}
Caecum											
methylamine	4.82	5.06	4.14	3.56	4.17	4.78	4.50	0.410	0.412	0.081	0.586
putrescine	8.22	7.26	8.74	4.29	7.10	6.92	11.39	1.722	2.689	0.354	0.601
cadaverine	2.71	2.21	2.34	1.67	3.58	1.65	5.03	0.621	0.914	0.751	0.101
histamine	5.71 b	4.19 a	4.45 a	4.11 a	5.37	4.22	4.67	0.393	0.622	0.048	0.304
tyrosine	2.19	1.58	1.85	1.26	2.74	1.74	1.82	0.343	0.381	0.186	0.679
spermidine	5.63	3.75	4.36	3.45	5.32	3.91	5.01	0.574	0.892	0.103	0.583
Colon											
putrescine	1.92	2.61	2.03	1.91	1.44	1.58	1.71	0.534	0.327	0.731	0.684

a, b – means in the same row with a different letters differ significantly at $P \leq 0.05$.

Table 5. Activity (U g^{-1}) of β-glucosidase and mucinase in the caecal digesta of chicks

Enzymes	C	IN_{10}			IN_{23}			SEM		P	
	0%	0.2%	0.4%	0.6%	0.2%	0.4%	0.6%	IN_{10}	IN_{23}	IN_{10}	IN_{23}
β-glucosidase	284	283	306	394	328	319	338	24.6	26.6	0.891	0.371
Mucinase	106	116	125	166	118	110	126	13.2	11.1	0.373	0.935

Table 6. Relative amount of selected bacterial populations in the caecal digesta of chicks (arbitrary units)

	IN_{10}				IN_{23}			SEM		P	
	0%	0.2%	0.4%	0.6%	0.2%	0.4%	0.6%	IN_{10}	IN_{23}	IN_{10}	IN_{23}
E. coli	0.40	0.35	0.46	0.19	0.30	0.29	0.35	0.051	0.038	0.279	0.740
Clostridium spp.	0.95	0.91	0.94	0.90	0.92	0.95	1.01	0.035	0.038	0.951	0.870
Lactobacillus spp.	0.64	0.65	0.52	0.61	0.52	0.79	0.70	0.040	0.053	0.746	0.319
Bifidobacterium spp.	below detection										

Relative amounts of *E. coli*, *Lactobacillus* spp. and *Clostridium* spp. in caecal digesta are shown in Table 6. Supplementation of diets with different levels of inulin, regardless of DP, did not affect significantly selected bacterial populations. *Bifidobacterium* spp. were not detected in the caecal digesta of chickens.

Discussion

Inulin and FSO share the basic common characteristics of dietary fibre such as plant origin, resistance to digestion and fermentability in the large intestine (Roberfroid, 2000). In several studies, the effects of oligosaccharides on microbial population and immune system of gastrointestinal tract of chickens were investigated (Kim et al., 2011; Alloui et al., 2013; Mookiah et al., 2014; Sugiharto, 2014). Huyghebaert et al. (2011) suggested that some prebiotics may be considered an alternative

to antibiotics. For example, inulin and FOS are thought to be preferable substrates for bifidobacteria, and, therefore, to promote their growth in the gut (Rada et al., 2001).

The main products of inulin fermentation are SCFA, predominantly acetic, propionic and butyric acids. Topping and Clifton (2001) reported that SCFA concentrations are always a net result of their production and absorption. The positive effect of inulin on the concentration of butyric acid in the caecal digesta of chickens was observed by Rehman et al. (2007, 2008) and Rebolé et al. (2010). They reported that concentration of this acid tended to be higher in broilers fed inulin. In contrast, Juśkiewicz et al. (2004) did not find differences in the caecal SCFA concentration in turkeys fed an inulin-supplemented diet. In our study, butyric acid did not exhibit any changes but propionic acid concentration was smaller in caecal digesta of birds fed diets containing 0.4% and 0.6% of IN_{10} compared to birds on diet with 0.2% level of IN_{10}. This may indicate a specific change in microbiota composition towards reduction of population of propionic acid-producing bacteria.

Intensive production of biogenic amines in the gut might lead to impaired nutrient utilisation and intestinal disturbance (Veldman et al., 1993). *Clostridium*, *Bifidobacterium* and *Bacteroides* have been shown to form amines in substantial quantities (Allison and Macfarlane, 1989). Besides, many of bacterial species responsible for monoamine production are able to produce lactate during rapid carbohydrates fermentation (Bailey et al., 2003). In the available literature there is no information about the effects of inulin on biogenic amines production in the gastrointestinal tract of poultry but studies on mammals indicated that inulin increases faecal concentrations of tryptamine, tyramine, phenylethylamine and cadaverine (Crawford et al., 2007). Terada et al. (1994) showed that lactosucrose decreased numbers of *C. perfringens* in chicken faeces and may be effective in decreased amines production in the colon. Our results are in agreement with Terada et al. (1994), because lower production of histamine and trend for lower methylamine concentration was observed in caecal digesta of chicks fed inulin but only with DP of 10.

The β-glucosidase is an enzyme responsible for the production of bacterial glucoside derivatives, which are assumed to be responsible for the protection against chemically induced cancer, but may also generate toxins (Roland et al., 1993). Mucinase is involved in the disruption of protective intestinal barrier composed of mucins that are utilized by bacteria as carbohydrate source (Shiau and Chang, 1983). A drop in the activity of bacterial enzymes in turkeys fed mannan-supplemented diets for 4 weeks, was observed by Juśkiewicz et al. (2006). In the same study, longer administration of mannans (8 or 16 weeks) did not change the activity between groups, but activity of particular enzymes was 10-fold higher after 16 weeks than after 4 or 8 weeks of feeding. In the present study, different levels of inulin, regardless of DP, had no impact on the β-glucosidase and mucinase activities in caecal digesta of chicks after 14 days of feeding.

Inulin may stimulate growth and activity of beneficial intestinal bacteria, such as *Bifidobacterium* and *Lactobacillus* and decrease pathogenic bacteria like *C. perfringens* and *E. coli* (Rebolé et al., 2010; Kim et al., 2011; Nabizadeh, 2012). Intestinal bacteria could also have positive implications for the ability of chickens digestion

and absorption of nutrients and thus for better performance of birds (Rehman et al., 2007). On the other hand, Zduńczyk et al. (2005) observed a decreasing tendency in the population of *Bifidobacterium* and *Lactobacillus* in turkeys fed diet with mannan-oligosaccharides addition. In the current research, we did not observe changes in *Lactobacillus* spp., *Clostridium* spp., *E. coli* and *Bifidobacterium* spp. populations in the caecal digesta due to feeding inulin. These data may indicate that the feeding period was too short, which along with birds' age, contributed to the lack of inulin effect. Similar reason was suggested also by Zhao et al. (2013).

Li et al. (2015) observed that oligofructose had a better prebiotic effect than inulin. Results of Juśkiewicz et al. (2004, 2006) also indicated that the large bowel microbiota of turkeys better utilized short-chain FOS than the long-chain inulin. In the present study, we observed only minor differences between inulin types of different DP on microbial activity in the large intestine of birds. As it was mentioned, fermentation efficacy is very low in the first week of a chicken's life, so it can be suggested that inulin was unable to affect microbiota activity.

The results of the current study indicate that feeding diet with 0.2%, 0.4% or 0.6% of inulin, regardless of degree of polymerization, has no relevant impact on the microbiota in the large intestine of two-week-old broiler chicks.

References

Allison C., Macfarlane G.T. (1989). Influence of pH, nutrient availability and growth rate on amine production by *Bacteroides fragilis* and *Clostridium perfringens*. Appl. Environ. Microbiol., 55: 2894–2898.

Alloui M.N., Szczurek W., Świątkiewicz S. (2013). The usefulness of prebiotics and probiotics in modern poultry nutrition: review. Ann. Anim. Sci., 1: 17–32.

Almagro-Moreno S., Pruss K., Taylor R.K. (2015). Intestinal colonization dynamics of *Vibrio cholerae*. PLoS Pathogens 11, e1004787.

Amit-Romach E., Sklan D., Uni Z. (2004). Microflora ecology of the chicken intestine using 16S ribosomal DNA primers. Poultry Sci., 83: 1093–1098.

Apajalahti J., Kettunen A., Graham H. (2004). Characteristics of the gastrointestinal microbial communities, with special reference to the chicken. World Poultry Sci. J., 60: 223–232.

Association of Official Analytical Chemists (AOAC). (2000). Methods of Analysis of AOAC International. 17th ed. AOAC, Arlington, VA.

Bailey S.R., Marr C.M., Elliott J. (2003). Identification and quantification of amines in the equine cecum. Res. Vet. Sci., 74: 113–118.

Barszcz M.,Taciak M., Skomiał J. (2011). A dose-response effects of tannic acid and protein on growth performance, caecal fermentation, colon morphology, and β-glucuronidase activity of rats. J. Anim. Feed. Sci., 20: 613–625.

Biggs P., Parsons C., Fahey G.C. (2007). The effect of oligosaccharides on growth performance, nutrient utilization and caecal microbes in young chicks. Poultry Sci., 86: 2327–2336.

Cao B.H., Karasawa Y. Guo Y. M. (2005). Effects of green tea polyphenols and fructooligosacharides in semi purified diets on broiler performance and cecal microflora and their metabolites. Asian-Australasian J. Anim. Sci., 18: 85–89.

Corrier D.E.A., Nisbet D.J., Scanlan C.M., Hollister A.G., Deloach J.R. (1995). Control of *Salmonella typhimurium* colonization in broiler chicks with a continuous-flow characterized mixed culture of cecal bacteria. Poultry Sci., 74: 916–924.

Crawford C., Sepulveda M.F., Elliott J., Harris P.A., Bailey S.R. (2007). Dietary fructan carbohydrate increases amine production in the equine large intestine: Implications for pasture-associated laminitis. J. Anim. Sci., 85: 2949–2958.

Desrouillères K., Millette M., Vu K.D., Touja R., Lacroix M. (2015). Cancer preventive effects of a specific probiotic fermented milk containing *Lactobacillus acidophilus* CL1285, *L. casei* LBC80R and *L. rhamnosus* CLR2 on male F344 rats treated with 1,2-dimethylhydrazine. J. Funct. Foods, 17: 816–827.

Ganguly S. (2013). Supplementation of prebiotics, probiotics and acids on immunity in poultry feed: a brief review. World Poultry Sci. J., 69: 639–648.

Huyghebaert G., Ducatelle R., Van Immerseel F. (2011). An update on alternatives to antimicrobial growth promoters for broilers. Vet. J., 187: 182–188.

Juśkiewicz J., Zduńczyk Z., Jankowski J. (2004). Selected parameters of gastrointestinal tract metabolism of turkeys fed diets with flavomycin and different inulin content. World Poultry Sci., 60: 177–185.

Juśkiewicz J., Jankowski J., Zduńczyk Z., Mikulski D. (2006). Performance and gastrointestinal tract metabolism of turkeys fed diets with different contents of fructooligosaccharides. Poultry Sci., 85: 886–891.

Kastner S., Perreten V., Bleuler H., Hugenschmidt G., Lacroix Ch., Meile L. (2006). Antibiotic susceptibility patterns and resistance genes of starter cultures and probiotic bacteria used in food. Syst. Appl. Microbiol., 29: 145–155.

Kaufmann P., Pfefferkorn A., Teuber M., Meile L. (1997). Identification and quantification of *Bifidobacterium* species isolated from food with genus-specific 16S rRNA targeted probes by colony hybridization and PCR. Appl. Environ. Microbiol., 63: 1268–1273.

Kim G.B., Seo Y.M., Kim C.H., Paik I.K. (2011). Effect of dietary prebiotic supplementation on the performance, intestinal microflora and immune response of broilers. Poultry Sci., 90: 75–82.

Li W., Zhang J., Yu Ch., Li Q., Dong F., Wang G., Gu G., Guo Z. (2015). Extraction, degree of polymerization determination and prebiotic effect evaluation of inulin from Jerusalem artichoke. Carbohyd. Polym., 121: 315–319.

Madrigal L., Sangronis E. (2007). Inulin and derivates as key ingredients in functional foods. Arch. Latinoam. Nutr., 57: 387–396

Mookiah S., Sieo C.C., Ramasamy K., Abdullah N., Ho Y.W. (2014). Effects of dietary prebiotics, probiotic and symbiotic on performance, caecal bacterial populations and caecal fermentation concentrations of broiler chickens. J. Sci. Food Agri., 94: 341–348.

Nabizadeh A. (2012). The effect of inulin on broiler chicken intestinal microflora, gut morphology and performance. J. Anim. Feed Sci., 21: 725–734.

Önal A., Tekkeli S., Önal C. (2013). A review of the liquid chromatographic methods for the determination of biogenic amines in foods. Food Chem., 138: 509–515.

Rada V., Duscova D., Maroune M., Peter J. (2001). Enrichment of *Bifidobacteria* in the hen caeca by dietary inulin. Folia Microbiol. (Praha), 46: 73–75.

Rebolé A., Ortiz L.T., Rodriguez M.L., Alzueta C., Treviño J., Velasco S. (2010). Effects of inulin and enzyme complex, individually or in combination, on growth performance, intestinal microflora, cecal fermentation characteristics, and jejunal histomorphology in broiler chickens fed a wheat- and barley-based diet. Poultry Sci., 89: 276–286.

Rehman H., Hellweg P., Taras D., Zentek J. (2008). Effect of dietary inulin on the intestinal short chain fatty acids and microbial ecology in broiler chickens as revealed by denaturing gradient gel electrophoresis. Poultry Sci., 87: 783–789.

Rehman H.C., Rosenkranz C., Bölem J., Zentek J. (2007). Dietary inulin affects the morphology but not sodium dependent glucose and glutamine transport in the jejunum of broilers. Poultry Sci., 86: 118–122.

Roberfroid M.B. (2000). Chicory fructooligosaccharides and the gastrointesinal tract. Nutrition, 16: 677–679.

Roland N., Nugon-Baudon L., Rabot S. (1993). Interactions between the intestinal flora and xenobiotic metabolizing enzymes and their health consequences. World Rev. Nutr. Diet., 74: 123–148.

Sauvant D., Perez J.M., Tran G. (Eds). (2004). Tables of Composition and Nutritional Value of Feed Materials. Wageningen Academic Publishers (The Netherlands) and INRA, Paris (France).

Shiau S.-Y., Chang G.W. (1983). Effects of dietary fiber on fecal mucinase and β glucuronidase activity in rats. J. Nut., 113: 138–144.
Smulikowska S., Rutkowski A. (2005). Recommended Allowances and Nutritive Value of Feedstuffs – Poultry Feeding Standards (in Polish). 5th Edition, The Kielanowski Institute of Animal Physiology and Nutrition, PAS, Jabłonna, Poland.
Sugiharto S. (2014). Role of nutraceuticals in gut health and growth performance of poultry. J. Saudi Soc. Agric. Sci., doi:10.1016/j.jssas.2014.06.001
Taciak M., Barszcz M., Tuśnio A., Bachanek I., Pastuszewska B., Skomiał J. (2015). Effects of type of protein and fibre fermented *in vitro* with different pig inocula on the short-chain fatty acids and amines concentrations. J. Anim. Feed Sci., 24: 235–243.
Tako E., Glahn R.P., Welch R.M., Lei X., Yasuda K., Miller D.D. (2008). Dietary inulin affects the expression of intestinal enterocyte iron transporters, receptors and storage protein and alters the microbiota in the pig intestine. Br. J. Nutr., 99: 472–480.
Terada A., Hara H., Sakamoto J., Sato N., Takagi S., Mitsuoka T., Mino R., Hara K., Fujimori I., Yumada T. (1994). Effect of dietary supplementation with lactosucrose on caecal flora, caecal metabolites, and performance in broiler chickens. Poultry Sci., 73: 1663–1672.
Thompson J.L., Hinton M. (1997). Antibacterial activity of formic and propionic acids in the diet of hens on Salmonellas in the crop. Br. Poultry Sci., 38: 59–65.
Topping D.L., Clifton P.M. (2001). Short-chain fatty acids and human colonic function: roles of resistant starch and nonstarch polysaccharides. Physiol. Rev., 81: 1031–1064.
Tsen H.Y., Lin C.K., Chi W.R. (1998). Development and use of 16S rRNA gene targeted PCR primers for the identification of *Escherichia coli* cells in water. J. Appl. Microbiol., 85: 554–560.
Valeri M., Rossi P.S., Kasendra M., Nesta B., Serino L., Pizza M., Soriani M. (2015). Pathogenic E. coli exploits SsIE mucinase activity to translocate through the musocal barrier and access to host cells. PLoS One. 10:e0117486.
Van der Wielen P.W.J.J., Biesterveld S., Notermans S., Hofstra H., Urlings A.P., Van Knapen F. (2000). Role of volatile fatty acids in development of the cecal microflora in broiler chickens during growth. Appl. Environ. Microbiol., 66: 2536–2540.
Veldman A., Veen W.A.G., Barug D., Wan Paridon P.A. (1993). Effect of α-galactosides and α-galactosidase in feed on ileal piglet digestive physiology. J. Anim. Physiol. Anim. Nut., 69: 57–65.
Vergauwen R., Van Laere A., Van Den Ende W. (2003). Properties of fructan: fructan 1-fructosyltransferases from chicory and globe thistle, two Asteracean plants storing greatly different types of inulin. Plant. Physiol., 133: 391–401.
Wang R.F., Cao W.W., Cerniglia C.E. (1996). PCR detection and quantitation of predominant anaerobic bacteria in human and animal fecal samples. Appl. Environ. Microbiol., 62: 1242–1247.
Windey K., De Preter V., Verbeke K. (2012). Relevance of protein fermentation to gut health. Mol. Nutr. Food Res., 56: 184–196.
Zduńczyk Z., Juśkiewicz J., Jankowski J., Biedrzycka E., Koncicki A. (2005). Metabolic response of gastrointestinal tract of turkeys to diets with different level of mannan-oligosaccharide. Poultry Sci., 84: 903–909.
Zhao P.Y., Wang J.P., Kim I.H. (2013). Effect of dietary levan fructan supplementation on growth performance, meat quality, relative organ weight, cecal microflora, and excreta noxious gas emission in broilers. J. Anim. Sci., 91: 5287–5294.
Zhu X.Y., Zhong T., Pandya Y., Joerger R.D. (2002). 16S rRNA-based analysis of microbiota from cecum of broiler chickens. Appl. Environ. Microbiol., 68: 124–137.

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