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Original article

Alternative for improving gut microbiota: use of *Jerusalem artichoke* and probiotics in diet of weaned piglets

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Abstract

The aim of the study was to determine the effect of Jerusalem artichoke and probiotics on defence activity of intestinal cells of weaning pigs. One hundred eighty piglets (7 weeks old) were fed with basal feed supplemented with *Jerusalem artichoke*, *Lactobacillus reuteri* and *Pediococcus pentosaceus*. After 5 weeks, the piglets were slaughtered and the gastrointestinal contents and intestine samples were taken for analysis. Results demonstrated that in pigs fed basal diet with both probiotics and Jerusalem artichoke (5% of basal diet) (T3 group) had less (P<0.05) faecal *Enterobacteriaceae* microorganisms and coliforms and had more (P<0,05) faecal *Lactobacillus* than in pigs from other groups. Increase by 2% of *Enterobacteriaceae* and *E.coli* levels were seen only in control piglets (T1 group). *E.coli O157* was found at the closing stage in the piglets fed basal diet with only Jerusalem artichoke powder (T2 group), but *Salmonella enteritidis* – only in T1 group. In jejunum of T2 group piglets, large deterioration of crypts, a moderate inflammation process and plasmocytes were seen, but in jejunum of T3 group piglets – branching of apical surface of villi, moderate degeneration and mitosis of enterocytes were observed. A moderate number of apoptotic cells in T2 group was found mainly in colon inflammation cells and plasmocytes, but for T3 group piglets – both in jejunum enterocytes and migrating cells. Our study indicated that β-defensin 2 and 3 expression in jejunum and colon segments were incresed in T1 and T2 groups. Findings suggest that feeding with probiotics and Jerusalem artichoke significantly improves the microbial contents, defence and regeneration processes in the intestine of pigs.

Key words: feed, pigs, immunohistochemistry, *Lactobacillus reuteri*, *Pediococcus pentosaceus, Jerusalem artichoke*

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Introduction

Farm animals are often subjected to environmental stress (management methods, diet, etc.) which can cause the intestinal ecosystem imbalance and could become a risk factor for pathogen infections. In commercial pig production, most stress factors are related to weaning and post-weaning periods (separation from the sow, end of the lactation immunity, early and critical transition from milk to a diet based on plant polysaccharides, moving of animals to a production farm). These periods are characterized by an immediate, but transient, drop in feed intake impairing growth performance of the animals. All these factors can negatively disturb the immune function and the equilibrium of intestinal microbiota in pigs (Modesto et al. 2009), leading to increased susceptibility to gut disorders, infections and diarrhoea (Gaggfa et al. 2010).

Prebiotics are non-digestible feed ingredients that favourably affect the host by stimulating the growth and activity of specific bacteria groups, mostly in the colon (Maxwell et al. 2004). The dominant prebiotics are fructo-oligosaccharide products (FOS, oligofructose, inulin). They are presumed to act by binding and removing pathogens from the intestinal tract and stimulating the immune system (Spring et al. 2000).

Lactic acid bacteria are the predominant microbes that can enhance host immunity and increase resistance to disease. Probiotics act mostly in the upper compartments of the gastrointestinal tract; while prebiotics are supposed to be used as substrates for potentially beneficial bacteria in the hind gut of monogastric animals. Therefore, the association of a specific prebiotic and probiotic may have a synergetically positive effect on intestinal microflora (Nemcová et al. 1999).

Intestinal epithelial cells must coexist with a high density of diverse bacteria. Protection against these bacteria exists on multiple levels – the impermeability of the intestinal epithelial barrier and serving as a protective barrier (the epithelium plays an active role in the intestinal immune response through its secretion of inflammatory cytokines, chemokines and antimicrobial peptides (Stadnyk 2002). Defensins are a class of antimicrobial peptides exerting their effect by damaging the bacterial cell membrane (Ganz 2003).

Although it is clear that probiotics have some effects on microbiota, little is known about action of probiotics and inulin-type-fructans on the defence activity of intestinal cells. In the present study, a number of important characteristics of a supplement composed of two probiotics (*Lactobacillus reuteri*, *Pediococcus pentosaceus*) and *Jerusalem artichoke* (Ja) were evaluated by examining the changes of gut microbiota and reactivity of intestine cells. The aim of this study was to determine the effect of *Jerusalem artichoke* and probiotics on weaning pigs gut microbiota and intestinal morphofunctionality.

Materials and Methods

Animals and experimental design

The experiment was conducted at the commercial pig farm (Jelgava, Latvia). The protocols of the experiments were approved by the Ethical Committee of the Ministry of Agriculture of Latvia. 180 helmint-free weaning piglets were used (30 animals in each group) from commercial line (Landrace x Large White), 28 days of age and with a body weight 7.8 ± 0.11 kg. The experimental design was conducted in randomized selection of animals, with six feeding trials and two replications. Prebiotics and/or probiotics were administered with basal diet once a day for 5 weaks. The experimental diets can be seen in Table 1.

The commercial basal diet was formulated following NRC-recommended (1998) feeding standarts. Powders from *Jerusalem artichoke* tubers (containing 45% of inulin), *Lactobacillus reuteri* (dose $1x10^9$ CFU/g) and *Pediococcus pentosaceus* (dose $1x10^9$ CFU/g) are commercially available. Powder of *Jerusalem artichoke* is made from cultivated plants in Latvia.

Microbiological analytical procedures

For the evaluation of microbiological parameters, fecal samples were collected from the rectum with sterile swabs at the beginning (1^{th} day) and at the end of the experiment (35^{th} day). One g of feces was taken from each sample and serially diluted 10-fold with sterile physiological saline for microbial counting. One ml of the appropriate dilution was plated on to different solid selective media employed for the quantification of different species.

Total lactobacilli and *Enterobacteriaceae* counts were determined as described by van Winsen et al. (2001). *Enterobacteriaceae* were cultured on plates with VRBG (Biolife, Italy) solid medium (24 ± 2 h at 37°C). Presumptive *Enterobacteriaceae* isolates were confirmed in accordance with international standard LVS ISO 21528-2:2007. For total lactobacilli counts, lactobacilli were cultured on Elliker broth (Sigma Aldrich, France) with Agar Bios Special LL (Biolife, Italy) (48 ± 2 h at 42°C). For detection of *E. coli* O157 Chromogenic *E.coli* Agar (Biolife, Italy) was used.

Table 1. Feeding trials of weaning piglets.

Experimental groups	Feeding diets
T1	basal diet (BD)
T2	basal diet + 3% of *Jerusalem artichoke* powder
T3	basal diet + *Lactobacillus reuteri* (0.5 g/day/piglet) + *Pediococcus pentosaceus* (0.5 g/day/piglet) + 5% of *Jerusalem artichoke* powder
T4	basal diet + *Lactobacillus reuteri* (0.5 g/day/piglet) + *Pediococcus pentosaceus* (0.5 g/day/piglet) + 3% of *Jerusalem artichoke* powder
T5	basal diet + *Lactobacillus reuteri* (1 g/day/piglet) + 3% of *Jerusalem artichoke* powder
T6	basal diet + *Pediococcus pentosaceus* (1 g/day/piglet) + 3% of *Jerusalem artichoke* powder

Plates were cultured for 24 ± 2 h at 37°C. The presence of *Salmonella* spp. was determined in accordance with international standards LVS EN ISO 6579/A1 and ISSO 6579:2002/Amd 1:2007. *Salmonella* at species level were identified using gram-negative kits of BBL CRYSTAL Identification System (BD, US). *Campylobacter* spp. was isolated in accordance with Manual of Clinical Microbiology (Murray et al. 2007).

All bacterial counts are expressed as log10 colony-forming units per gram (CFU/g).

PCR

At the end of experiment, five animals (randomly selected) from each group were slaughtered after electric desensitization and samples of jejunum and colon content was removed aseptically for examination with PCR. Tissue samples were placed in sterile plastic tubes with raps. Biopsies and stools were stored at -80°C until DNA extraction.

For DNA extraction 100 mg of pig feces or intestinal mucosa scrapings were taken and washed in 1 mL TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.6) by vortexing and centrifugation. For DNA extraction 2 different methods were applied. First method includes cell lysis by SDS (sodium dodecyl sulfate) and proteinase K with accordance to Owen and Borman (1987). For DNA precipitation 1/25 volumes of 5 M NaCl and 2 volumes of 96% ethanol were added. DNA pellets were washed with 1 ml of 70% ethanol. Second method includes cell lysis with CTAB (cetyl trimethylammonium bromide) solution (Lipp et al. 1999). Two different DNA extraction methods were applied to all biological materials tested. Such approach results in two independent DNA preparations for each sample, minimizing occasional possibility of contamination or poor DNA recovery from some microorganisms.

Finally, DNA was dissolved in sterile deionised water. Quality and quantity of extracted DNA were tested spectrophotometrically (UV 260/280/230 nm) and with electrophoresis in agarose gel. For PCR DNA solutions with concentration 100 ng/ µL were prepared. Extracted DNA was amplified by a nested PCR specific for *L. intracellularis*, genomic DNA, as previously described (Jones et al. 1993). PCR second reaction will amplify a 270-base pair (bp) DNA fragment of p78 sequence. In all experiments negative control samples without DNA template were subjected to PCR amplification. The PCR products were subjected to the electrophoresis in 1.5% agarose gels in 0.5x TAE buffer (20 mM Tris-acetate, 0.5 mM EDTA), stained with ethidium bromide and visualized over UV light. For size evaluation of DNA fragments GeneRuler™ 100 bp Plus DNA Ladder (Fermentas, Lithuania) was used.

Histology and immunohistology

The sections of approximately 2.0 cm were aseptically removed from the jejunum and colon of slaughtered pigs, washed in distilled water and fixed in 10% formaldehyde solution. Multiple 6 µm-thick sections of the paraffin-embedded piglet intestine were stained with haematoxylin and eosin (HE), examined for immunohistochemistry (Hsu et al. 1981).

Prior to immunostaining, sections were deparaffinized and rehydrated. Sections were processed in microwave for 20 min in 4% citrate buffer (pH 10), quenched for 10 min with 3% H_2O_2 for blocking endogenous peroxidase activity, rinsed in phosphate-buffered saline (pH 7.4), pretreated with a nonimmune goat serum for 10 min for blocking of nonspecific antibody binding and then incubated for 2 h with the primary antibodies.

The primary antibodies used in immunohistochemistry were goat polyclonal antibodies specific

Table 2. The presence of microorganisms in fecal contents.

Items	Experimental groups					
	T1	T2	T3	T4	T5	T6
Enterobacteriaceae (log_{10} CFU/g)						
1th day	7.9+0.78					
35th day	8.1+0.49*	7.7+0.14	7.2+0.21	7.5+0.14	7.4+0.14	7.6+0.07
E.coli (log_{10} CFU/g)						
1th day	7.8+0.28					
35th day	8.0+0.35*	7.7+0.21	6.9+0.14*	7.1+0.07	7.2+0.07	7.3+0.14
E. coli O157						
1th day	NF	NF	NF	NF	NF	NF
35th day	NF	NF	Found	NF	NF	NF
S. enteritidis						
1th day	NF	NF	NF	NF	NF	NF
35th day	NF	NF	NF	NF	NF	Found
LAB (log_{10} CFU/g)						
1th day	8.2+0.14					
35th day	8.1+0.12*	8.7+0.22	9.5+0.14	9.2+0.14	9.0+0.07	8.9+0.29
Lawsonia intracellularis	NF	NF	NF	NF	NF	NF

* value ($P<0.05$) differ significantly
CFU – colony forming units
LAB – Lactic-Acid Bacteria
NF – not found

for β-defensin 2 (BD2) (dilution 1:100, code: AF2758, R&D System, DE) and rabbit polyclonal antibodies specific for β-defensin 3 (BD3) (dilution 1:100, code: LS-BP86/8279, LifeSpan Bioscience).

Immunoreaction was visualized by the avidin-biotin (LSAB) immunoperoxidase method using an LSAB kit (DakoCytomation, DK), and DAB (diaminobenzidine) solution (Dako, DK) was used as chromogen, while hematoxylin was used as the counterstain.

TUNEL reaction was used for detection of apoptosis (Negoescu et al. 1998).

Selected intestine tissue sections also were stained with the Warthin – Starry stain (Bio-optica, IT) in accordance to instruction of manufacturer.

Statistical analysis

Values of microbial parameters are given as the means ± standart error (SE). Significance was tested by applying Student's t-test. Probability values of less than 0.05 ($P<0.05$) were considered significant. The bacterial concentrations were transformed (log) before statistical analysis.

Semi-quantitative analysis was used to estimate proportions of immunopositive cells in intestines (Pilmane et al. 1995). The designations were as follows: (+) – few positive cells; (++) – moderate and (+++) – numerous positive cells in the view field.

The apoptotic index was calculated from the number of apoptotic cells expressed as a percentage of 100 cells counted.

Results

Throughout the feeding trials all the piglets were healthy (without signs of diarrhoea or loss of appetite).

Bacterial populations

Table 2 shows the effect of the tested prebiotics and probiotics on the LAB (Lactic-Acid Bacteria) counts in fecal samples. The mean values of LAB showed significant difference between trials pigs and at day 35 they were from 8.1 to 9.5 log_{10} CFU/g, on the average. However, in T3 group piglets, LAB level after 35 days of intake significantly ($P<0.05$) increased (15%) in comparision with the level at trial day 1. In T1 group LAB level was significantly ($P<0.05$)

Table 3. Morphological findings in the jejunum and colon of supplement-fed weaned pigs.

Items / groups	T1		T2		T3		T4		T5		T6	
	jej	col	jej	col	jej	col	jej	col	jej	col	jej	col
H/E												
villi necrosis			+++		++		+		+		+	
inflammation cells	+	++	++	++	+	+	+	+	+	+	+	+
plasmocytes	+		++		+		+		+		+	
mitosis	+		+		++		+		+		+	
degeneration of cripts		+		+		++		+		+		+
Apoptosis												
enterocytes	++		+		++				+		+	
inflammation cells	+	+	+	++	++	+	+	+		+		+
cripts			+						+			
fibroblasts	+	+	+	+		+						+
apoptotic index (%)	25	21	21	27	34	38	13	16	8	17	11	16
β-defensin 2												
enterocytes		+++	++									
inflammation cells		+		++								
fibroblasts	+++		++		+		+		+		+	
β-defensin 3												
enterocytes	+		++									
goblet cells	+		+									
inflammation cells	+		+						+		+	
fibroblasts	+		++		+		+		+		+	
Intracellular inclusions	+++		+++		++		+		+		+	

jej – jejunum,
col – colon

lower than in other groups. With regard to mean levels of *Enterobacteriaceae* and *E.coli* in samples analysed, there were no essential differences observed between trial pigs with the exception of T3 group animals. In this group, the number of microorganisms of genera *Enterobacteriaceae* at the end of trial were decreased by 8%, but *E.coli* – decreased by 11%. Increase by 2% of *Enterobacteriaceae* and *E.coli* levels was seen only in T1 group.

Pathogenic microorganism *E.coli O157* was found at the closing stage of the T2 group piglets, but *Salmonella enteritidis* – only in T1 group of above-mentioned stage. Since cultivation and estimation of some microorganisms (such as *Lawsonia spp.*) with classic microbiological methods is difficult, detection of microorganisms by amplification of their specific DNA sequences with polymerase chain reaction (PCR) is need widely. *Campylobacter coli* and *L. intracellularis* were not found in any group of piglets.

Intestinal morphology

The morphology of the small intestine differed between the supplement-fed weaning pigs and the controls (Table 3). Weak focal villi necrosis, separate plasmocytes, inflammation and mitotic cells, but high extrusion process on the surface of jejunal enterocytes were observed in trial pigs. In T1 group animals villi were slim and the small intestinal mucosa revealed no histopathological changes. More histopathological changes were seen in T2 and T3 groups pigs. In T2 group, a distinct degeneration process of crypts, moderate inflammation process and plasmocytes were seen, but in T3 group – branching of the apical surface of villi, moderate degeneration and mitosis of enterocytes were observed. Villi had broken tip areas with large holes and surfaces free of microvilli as compared with T1 group animals.

In colon, only a weak degeneration of crypts

and inflammation were found in piglets with exception of T1, T2 groups (moderate inflammation process) and T3 group (moderate degeneration of crypts).

Few scattered apoptotic cells were seen in jejunal enterocytes and connective tissues, however in enterocytes of the T1 pigs this process was moderate. Apoptotic epithelial cells were present over the entire length of the villi, but concentrated at the top of the villi. In T2 group, a moderate number of apoptotic cells was found mainly in colon inflammation cells and plasmocytes, but in T3 group of piglets, both, in jejunum enterocytes and migrating cells. The apoptotic index was higher in the colon of T2 group pigs (27%) and in jejunum and colon of T3 group animals, 34% and 38%, respectively.

Table 3 presents the expression of β-defensins (BD) roughly in all intestinal cells that showed a signal above the backgroung. Our investigation indicated that BD2 expression in jejunum and colon segments were incresed in T1 and T2 groups that indicated an increasing amount of intestinal bacteria compared with other experimental pigs. BD2 and BD3 expressions were seen mainly in enterocytes, inflammatory cells and in connective tissue cells.

Warthin-Starry stained sections of jejunum and colon revealed roundish organisms in the cytoplasm of the villi enterocytes of all experimental piglets, but a significant number was seen only in T1 and T2 groups.

Discussion

The gastrointestinal tract (GIT) is unique, as it represents the largest area of the body constantly exposed to microorganisms. Weaning stress is an important factor which can destroy the balanse of intestinal microbiota (Blomberg et al. 1993). Feed additives such as prebiotics or probiotics can modulate the gut microflora improving the performance of livestock. The evaluation of Lactic-Acid Bacteria (LAB) and fructo- oligosaccharides (FOS) for their potential use as probiotics and prebiotics in farm animals is still increasing (Denli et al. 2003, Bogovič et al. 2004).

Oligosaccharides are usually defined as prebiotics that can selectively stimulate the growth of health-promoting bacteria (Gibson and Roberfroid 1995). The results of the current study have shown that feeding orally just 3% of *Jerusalem artichoke* powder did not sensibly alter microbiota in GIT of pigs in comparison with other trial groups. It is established, that oligosaccharides incorporated in swine diets at levels ranging from 5 to 40 g/kg per diet have resulted in a variet, but generally not significant, effect regarding beneficial modulation of microbial populations detected in feces of swine (Mikkelsen et al. 2003) and the supplementation of piglets a diet with 40 g FOS/kg had no effect on anaerobic bacteria and enterobacteria in feces of pigs (Flickinger and Fahey 2002). As to numbers of *E.coli*, no differences were detected altogether (Tako et al. 2008).

Probiotics can beneficially affect the host animals by improving their intestinal microbial balance (Fuller 1989). *Lactobacillus* is deemed a target organism because of their potential to inhibit the growth of pathogenic bacteria (Paton et al. 2006). In the current study, LAB number in control group was reduced by 1%. Konstantinov et al. (2006) also indicated that after weaning of pigs, lactobacilli concentrations decreased. It is generally accepted that lactobacilli are important to maintain good intestinal health because of their ability to control potentially pathogenic groups, such as *E.coli* (Blomberg et al. 1993, Shu et al. 2001). Tako et al. (2008) indicated that *Jerusalem artichoke* supplementation improved the gastrointestinal Lactobacillus population, but our results showed that feed supplementation only with 3% of *Jerusalem artichoke* were less effective in increasing of LAB counts than other diets. Apparently, such amount of LAB in the gut are beneficial for pathogenic microorganisms, because *E.coli* O157 in experimental T2 group and *S.enteritidis* in control (T1) group were found, that led to a higher incidence of diarrhea. The oligosaccharide has been shown to be effective in reducing the adhesion of certain strains of enteropathogenic *E.coli* to intestinal cells but not to nontoxigenic, commensal flora (Rhoades et al. 2006); thus, the decrease in total *E.coli* counts may have resulted from the decreased number of enteropathogenic *E.coli*. The changes in GIT microbiota of the T1 group pigs can be measured by investigation of the bacterial fermentation activities. Authors (Mathew et al. 1994, Konstantinov et al. 2006) found that in the intestine of weaned piglets the decreased LAB population and increased *E.coli* counts coincided with decreased volatile fatty acids and increased lactate concentrations.

Significant impact on the gut microbiota was found only in case of the synergistic effect of *Jerusalem artichoke* and probiotics. The results have shown that such combination could reduce levels of enteric pathogens in fecal samples compared with the control group, because *Jerusalem artichoke* with probiotics supplementation has successfully increased the population of LAB and decreased the counts of *Enterobacteriacea* and, ecpecially, the number of *E.coli* in fecal samples of pigs. Several studies *in vitro* in pigs found that *Lactobacilli* can compete with pathogens for adhesion sites in the gut (Spenser and Chesson 1994), however in our study difference of

microbial counts between groups fed *Lactobacillus* and *Pediococcus* were not seen. The most signifficant reduction of enteric bacteria was observed in T3 group piglets, that indicated the synergistic effect of probiotics. Other scientists (Ly et al. 1995, Nemcová et al. 1999) observed that 3g FOS/day and 2g *Lactobacillus paracasei* not only significantly reduced *Enterobacteriaceae*, but also essentially increased total aerobes, anaerobes and lactobacilli. Commonly, reduction of pathogenic microorganisms in gut of animals can be explained by the fact that LAB reduces pH in the luminal contents, which can inhibit the proliferation of pathogenic bacteria. In addition, acetic acid excreted by LAB and H_2O_2 may be toxic to coliforms, Salmonella and Clostridia *in vitro* (Nousiainen and Setälä 1998).

The structure of the intestinal mucosa can reveal certain information about gut health. In the present study, we observed that diet supplementation with prebiotic (*Jerusalem artichoke*) alone, causes severe alterations in the morphology of jejunal villi and is associated with higher tissue turnover. It is established that the presence in the lumen of high viscosity digesta may increases the rate of villus cell loss, leading to villus atrophy (Montagne et al. 2003), although it is observed that inulin supplementation altered neither mucosal morphology nor epithelial proliferation (Meijer et al. 2000). Cell mitosis in the intestines of trial pigs indicated that the function of the intestinal villi is activated (Yasar and Forbes 1999). In addition, in T2 group pigs, pathogenic *E. coli* O157 was found that could be as causal agent for increasing of inflammation cells in jejunum villi and colon, because the prebiotics stimulate the growth and/or metabolic activity of different bacterial species, including species that are both potentially harmful and beneficial (Maczulak et al. 1993).

Small intestinal cell turnover is closely associated with cell extrusion from the villous apex via apoptosis and cell sloughing or invagination (Montagne et al. 2003). Apoptotic index in the colon of animals fed only wih *Jerusalem artichoke* (T2 group) and in the jejunum/colon of T3 group pigs was significantly higher. This led the authors to hypothesis that the impact on evolution of apoptotic process was caused by the high number of enteric microorganisms, because in enterocytes of jejunum of the pigs from groups T2 and T3 the highest number of intracellular inclusions was seen despite the fact that the presence of the specific microorganism *Lawsonia intracellularis* was not found in any of the intestinal mucosa samples tested. According to observations of other scientists apoptosis was increased in the normal mucosa of the probiotic group rats (Femia et al. 2002) and inulin supplementation do not alter cell death (Meijer et al. 2000) Our findings reflect a more efficient distal absorption of enteral diets, resulting in increased exposure in the proximal intestine. This observation indicates that probiotics in feed preserve villi architecture. For example, the probiotic *Lactobacillus* may modulate the intestinal immune response through the stimulation of certain cytokine secretion by epithelial cells (Delcenserie et al. 2008).

Intestinal epithelial cells regulate the intestinal immune response (Hase and Ohno 2006) and defensins are one of the major families of antimicrobial peptides (Lehrer and Ganz 2002). It was demonstrated that probiotics enhance secretion of β-defensin (BD) (Delcenserie et al. 2008), however in our study only separate β-defensin positive connective tissue and inflammation cells were observed. Although in a healthy colon β-defensin 2 (BD2) and β-defensin 3 (BD3) are absent, low levels of BD2 and BD3 induction may result in a defective antimicrobial barrier function at the mucosal surface (Zilbauer et al. 2005). Our finding about a positive relation between enteric pathogens and intensity of defensin expression in intestine cells of pigs testifies to the stimulating role of defensin on cell proliferation as a compensatory mechanism that agrees with findings of other researchers (Zilbauer et al. 2005, Schlee et al. 2008). With confirmation of authors (Veldhuizen et al. 2008) that porcine BD2 was active against both gram-positive and gram-negative bacteria *in vitro*, *i.e.* to *E.coli*, we can explain correlation in our study between the significantly increased amount of β-defensins and pathogenic macroorganisms (enteropathogenic *E.coli* and *S.enteritidis*) in intestines. The observed expression of BD against intestinal microorganisms suggests that these peptides may play a certain role in limiting the infection.

In conclusion, the present study indicated that weaning could cause a reduction of LAB and increase a number of microbial populations in piglet intestines, such as *Enterobacteriaceae, E.coli* and *S.enteritidis*. The synbiotic action of *Lactobacillus reuteri* and *Pediococcus pentosaceus* with Jerusalem artichoke has the potential of inhibiting pathogenic microflora of gut. Our findings suggest that feed with the single supplement of *Jerusalem artichoke* significantly enhances the defence and regeneration processes in the intestine of pigs. Stimulation of β-defensin production in the intestine through dietary modulation (probiotics with lower dose (3%) of *Jerusalem artichoke*) could promote the intestinal health of pigs, thereby reducing the occurrence of infections in pig herds and the subsequent use of antibiotic treatment.

This is the first demonstration of β-defensin 2 and β-defensin 3 expression in the intestinal epithelium of porcine jejunum and colon fed with supplement of

probiotics (*Lactobacillus reuteri*, *Pediococcus pentosaceus*) and *Jerusalem artichoke* concentrate.

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