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Differences in the Development of the Small Intestine Between Gnotobiotic and Conventionally Bred Piglets

Soňa Gancarčíková
University of Veterinary Medicine and Pharmacy, Košice
Slovakia

1. Introduction

The health quality of human population is strongly connected to the decrease of environmental burden and increase of quality and safety of food. The production of high-quality and safe food and materials of animal origin is conditioned by the good health of raised animals. Diseases of the gastrointestinal tract can be considered the most important health and economic problem of rearing young animals, since they may cause extremely high losses due to morbidity, mortality, cost of treatment and weight loss. At an early age, diseases debilitate the animal organism and cause delays in development which can subsequently become evident as health problems and decreased productivity. For this reason, it is extremely important to ensure optimum development of the digestive tract in young animals. These relations are determined by digestive juice and enzyme secretion, morphological development and microbial colonization of the digestive tract as well as by absorption capacity of the latter. The pig gut is exposed to a variety of stress factors particularly in the early postnatal period and just after weaning. This is the period of significant growth, morphological changes and maturation of the gastrointestinal tract (Godlewski et al., 2005; Trahair & Sanglid, 2002; Xu, 1996). Prior to birth, the alimentary tract is exposed to substances from the ingested amniotic fluid which seems to be of importance to its development (Trahair & Sanglid, 2002). The colostrum, however, differs from the amniotic fluid by the density of nutrients and high immunoglobulin, enzyme, hormone, growth factor and neuroendocrine peptide levels. Widdowson & Crabb (1976) were the first to demonstrate the effect of the colostrum upon development of the alimentary tract by comparing the colostrums-suckling piglets with watered animals. Maternal colostrums contained high levels of several hormones and growth promoting peptides like insulin, epidermal growth factor (EGF), insulin-like growth factor-I and II (IGF-I and II), transforming growth factor-β (TGF- β), glucagon-like peptide-2 (GLP-2) and leptin. It was proved that colostral growth factors play an important role in the postnatal development of the digestive tract in newborn animals (Guilloteau et al., 2002; Xu, 1996). During the several initial days of life of newborns, their small intestine increases its weight by about 70%, length by approx. 20%, diameter by 15%. Its absorption area increases by about 50% during the first postnatal day and by 100% during the first 10 postnatal days (Marion et al., 2003; Xu, 1996). A large luminal surface area with optimal enterocyte functional maturity is

important to young growing pigs so they may attain maximum digestive and absorptive capability. Consequently, suboptimal or adverse environmental factors, influencing the morphological development of intestinal tissue, may have critical functional consequences for the young growing pig. The marked and abrupt morphological responses to weaning in the small intestine, characterized by the transformation from a dense finger-like villi population to a smooth, compact, tongue-shaped luminal villi surface may indicate critical consequences for the young pig digestive capacity and subsequent use of nutrients during the starter phase (Skrzypek et al., 2005). The changes at weaning which include shortening of villi, hyperplasia of crypts, decrease in absorption capacity and certain loss of carbohydrate activity may, in combination with changes in the number and type of enterobacteria, induce various degree of post-weaning diarrhoea (Pluske et al., 1997). By now, the prevention and therapy of diseases of sucklings and weanlings was implemented by means of synthetic substances, which enormously burden not only the organisms, but also the living environment as a whole. The extensive use of antibiotics has increased the risk of development of resistance in human and animal pathogens and chemical residues in meat of animals. In progress is the research and development of new methods of biotechnological and natural character that with their complex influence will maximally make efficient the prevention of diseases of animals by the stabilisation of physiological function of biological barriers of the gastrointestinal tract ecosystem. Biological barriers of digestive tract represent the prime and basal protection of organism from negative impacts of external and internal environment, and therefore it is possible to decrease a health risk by its sophisticated modulation. The indigenous microbiota suppresses colonization of incoming bacteria by a process named colonization resistance that is a first line of defence against invasion by exogenous, potential pathogenic organisms or indigenous opportunists. Beneficial microbiota prevent bacterial colonization by competing for epithelium receptors and enteric nutrients, producing antimicrobial compounds such as bacteriocins and metabolizing nutrients to create a restrictive environment which is generally unfavourable for the growth of many enteric pathogens (Bomba et al., 2002; Marinho et al., 2007). Probiotics as natural bioregulators assist the maintenance of the homeostasis of the gastrointestinal tract ecosystem and, during the critical periods of animal life, can play an important role in prevention of diarrhoeic diseases of dietetic and bacterial origin (Bomba et al., 2002; Marinho et al., 2007). Gastrointestinal microflora may be affected by adding probiotic micro-organisms of genera *Lactobacillus*, *Bifidobacterium* (Bomba et al., 2002), *Bacillus*, *Enterococcus* and *Streptococcus* (Scharek et al., 2005) to feed or by their combinations (Bomba et al., 2002; Mathew et al., 1998). Enterococci belong to those lactic-acid bacteria which inhabit human and animal intestines (Devriese et al., 1991). It was observed that *Enterococcus faecium* prevents adherence of enterotoxigenic *Escherichia coli* K 88^{+} to the surface of intestinal mucosa of piglets (Scharek et al., 2005). In terms of exactitude and interpretability of results, gnotobiotic piglets are an ideal experimental model for the study of digestive processes and their development. The presence of normal microflora influences the structure of the host intestinal mucous membrane, its function and short-chain fatty acids (SCFAs) production. By means of gnotobiotic conditions, we excluded the influence of the normal microflora and sow's milk. The changes in the small intestine, observed under the specific controlled conditions, were compared to the development of the gut in conventionally bred piglets.

The aim of the study was to evaluate the effects of piglet´s age and diet (natural feeding, artificial feeding and gnotobiotic conditions) on the development of microflora, production of short-chain fatty acids (SCFAs), postnatal morphological development and

disaccharidase enzymes activity in the small intestine in piglets reared under the sow, piglets fed on milk replacement, as well as in gnotobiotic piglets.

2. Materials and methods

The experiments on growing and weaned piglets were carried out at the Institute of Microbiology and Gnotobiology, University of Veterinary Medicine and Pharmacy, Košice, Slovakia. The State Veterinary and Food Administration of the Slovak Republic approved the experimental protocols and the animals were handled and sacrificed in a humane manner in accordance with the guidelines established by the relevant commission.

2.1 Animal, housing and diets

2.1.1 Gnotobiotic piglets - 1st experiment

The experiment was carried out in 4 gnotobiotic units, each consisting of reserve, waste and rearing isolator (Velaz s.r.o., Prague, Czech Republic). All experimental materials, including milk substitute, distilled water, saline solution and glass and metal materials were sterilised by autoclaving at 121°C and pressure 1.3 MPa for 30 minutes and cellulose wadding and other sanitary material was gamma-irradiated (Bioster, Veverská Bitýška, Czech Republic). The isolators were sterilized with a 2% solution of peracetic acid (36%, Merci s.r.o., Brno, Czech Republic), sealed for 24 hrs, and vented for a minimum of 72 hrs prior to placing pigs inside. Isolators were maintained under positive pressure, the filtering unit consisting of a fan with preliminary EU 3 filter and two-stage filtering chamber (Velaz s.r.o., Prague, Czech Republic). The first stage of filtration consisted of a frame filter type KS-W, filtration class F 7, the second stage used a KS MIKRO S filter, filtration class H 13, for removing of microparticles. The vented air passed through a frame filter KS W/48, filtration class F 5. The filtration unit assured a minimum of 10 exchanges of air per hour at overpressure of 50-70 kPa and air flow 8-30 m^3.

The experiment was carried out on 18 gnotobiotic piglets of Slovak white × Landrace breed. Gnotobiotic sucklings were obtained using the method of open hysterotomy on day 112 of pregnancy. After opening the abdominal cavity and uterus the piglets were immediately transferred through a disinfectant bath containing 2% Incidur® (Ecolab GmbH & Co. OHG, Düsseldorf, Germany) into a hysterectomy box were they were subjected to preliminary treatment and then were placed into 1 of 4 gnotobotic rearing isolators. The floor of isolators was heated by electric underfloor heating system to ensure floor temperature of 34°C for new born piglets and 30°C for 7-14 days old piglets. The piglets were non-colostral and were fed autoclaved milk substitute (Sanolac Ferkel, Germany, in 1 kg dry matter: fat 18.0%, N-free extract 20.0%, lysine 1.7%, Ca 0.9%, P 0.7%, Na 1.0%, Mg 0.2%, fibre 1.5%, ash 10.0%, ME 17.5 MJ, vitamin A 50 000 IU, vitamin D_3 5 000 IU, vitamin E 100 mg, biotin 200 µg, Fe 100 mg, vitamin B_1 4 mg, vitamin B_2 4 mg, vitamin B_6 2 mg, vitamin B_{12} 20 µg, calcium pantothenate 10 mg, nicotinic acid 20 mg, folic acid 1 mg, vitamin C 100 mg, choline chloride 250 mg), diluted 1 : 5 with distilled water. The milk substitute was fed to piglets individually from a glass bottle six times daily (2, 6, 10, 14, 18, 22 h), *ad libitum*. A total of 18 gnotobiotic animals derived from 2 litters were divided into 4 isolators. From the first day of life, a probiotic strain of *Enterococcus faecium* isolated from non autoclaved milk substitute (Sanolac Ferkel, Germany) was administered continuously at a dose of 2 ml of inoculum;

1 ml contained 1 × 10^4 cfu (data analysed in the Laboratory of Gnotobiology). From the 5th day of life, autoclaved water was available to piglets *ad libitum* and they were fed irradiation-sterilized rations intended for early weaning of piglets. At the age of 28 days, the suckling piglets were weaned and fed irradiation-sterilized starter feedstuff *ad libitum* (OŠ-02®, Tajba Čaňa, Slovak Republic, in 1 kg dry matter: crude protein 180 g, fibre 45 g, lysine 11.5 g, methionine and cysteine 6.3 g, threonine 7.5 g, Ca 7 g, P 5.8 g, Na 1.5 g, Cu 10 mg, Zn 100 mg, Mn 30 mg, ME 13 MJ, vitamin A 8 000 IU, vitamin D_3 1 000 IU, vitamin E 20 mg, Fe 125 mg, vitamin B_2 3 mg, vitamin B_{12} 20 µg, choline 600 mg). A routine microbiological control of gnotobiotic isolators was performed throughout the experiment. Microbiological swabs were taken from isolator walls, surface of animals and from their rectum. The samples were cultivated in PYG medium (Imuna, Slovak Republic). The microbiological control was verified every day on TSA agar with 5% ram's blood (BBL, Microbiology systems, Cockeysville, USA).

2.1.2 Conventional suckled piglets - 2nd experiment

In the experiment, 24 piglets of both sexes (Large white breed x Landrace) from two litters were included. The pigs were housed in two pens, 12 piglets in each, equipped with automatic heating, forced ventilation and completely slatted floors. The suckling piglets had access to sows 6 times daily (2, 6, 10, 14, 18, 22 h.) and from day 5 onwards the animals were provided commercial mixed feed OŠ-01® (Tajba Čaňa, Slovak Republic, in 1 kg dry matter: crude protein 200 g, fibre 40 g, lysine 14 g, methionine and cysteine 6.3 g, threonine 9.1 g, Ca 8 g, P 6.7 g, Na 2 g, Cu 10 mg, Zn 100 mg, Mn 30 mg, ME 13.3 MJ, vitamin A 8 000 IU, vitamin D_3 1 000 IU, vitamin E 20 mg, Fe 125 mg, vitamin B_2 3 mg, vitamin B_{12} 20 µg, choline 300 mg) *ad libitum*. The piglets were weaned at 28 days of age, fed starter feedstuff *ad libitum* (OŠ-02®, Tajba Čaňa, Slovak Republic) and moved to 2 pens (375 x 165 cm) where three piglets were housed per pen. The temperature in the nursery was maintained at 32°C during the first week, and was gradually reduced to 25°C between weeks two and six. The animals had free access to water throughout the experiment (42 days).

2.1.3 Conventional replacer-fed piglets - 3rd experiment

The experiment included 26 piglets of both sexes (Large white breed x Landrace) from two litters. The experiment was carried out in two blocks, 13 piglets in each. The piglets were separated from the sow immediately after birth and had no contact with sow faeces. They were born naturally, were non-colostral, and were fed a commercial milk replacer diluted with distilled water 1:5 (Sanolac Ferkel, Germany), enriched by *Enterococcus faecium* 0.1 × 10^4 cfu/g of feed. Milk was given to piglets individually from a glass bottle 6 times daily (2, 6, 10, 14, 18, 22 h.), *ad libitum*. Starting from day 5, the suckling piglets were offered the same commercial mixed feed OŠ-01® (Tajba Čaňa, Slovak Republic) and were housed under the same hygiene conditions as those in the second experiment.

2.2 Experimental procedure

All pigs were sacrificed by intracardial euthanasia with 1 ml/kg BW T61® (Intervet International B.V. Boxmeer, The Netherlands). In the first experiment, three hours after birth and at the age of two and seven days, two piglets of each indicated age were sacrificed. Three piglets of each indicated age were sacrificed at the age of 14, 21, 28 and 35 days. In the course

of conventional experiments, three piglets from each group were sacrificed at 3 hours post partum and at 2, 7, 14, 21, and 28 days of age. In the second experiment the piglets were slaughtered also on days 35 and 42 of age. The gastrointestinal tract was immediately removed and divided into six segments as follows: stomach, three equal segments of the small intestine, caecum and colon. The total content of each segment was weighed, pH was immediately measured. Intestinal tissue (1 cm^2) were taken from the duodenum (5 cm distal to the orifice of the pancreatic duct) and the medial part of both the jejunum and ileum. The samples were fixed in 4% formalin solution for microscopic assessment of mucosal morphology. Sections of jejunum, ileum and caecum (1 g) were collected and processed for microbial counting and short-chain fatty acids (SCFAs) determinations were carried out in the contents from jejunum, ileum and colon. The intestinal segments (duodenum, jejunum and ileum) were rinsed thoroughly with ice-cold saline solution, opened lengthwise and blotted dry. The mucosa was scraped using a glass slide and immediately frozen in liquid nitrogen. Samples of mucosa were then stored at -70^{o}C until the analysis of digestive enzyme activities.

2.2.1 Microbiological analysis

For microbial analysis, about 1 g of samples (jejunum, ileum, caecum) was placed in a sterile polyethylene stomacher Lab Blender bag (Seward Medical Limited, London, UK) with 9 ml of sterile anaerobic diluent (0.4 g Na HCO_3, 0.05g L-cysteine HCl, 1 ml resazurine 0.1%), 7.5 ml mineral solution I (0.6% K_2HPO_4), 7.5 ml mineral solution II (1.2% NaCl, 1.2% $(NH_4)_2SO_4$, 0.6% KH_2PO_4, 0.12% $CaCl_2$, 0.25% $MgSO_4$ and 84 ml distilled water, pH 6.8) and stomached (Stomacher Lab Blender 80, Seward Medical Limited, London, UK) for 5 min under a CO_2 atmosphere. A series of 10-fold dilutions (10^{-2} to 10^{-8}) were made in the same diluents. From appropriate dilutions, 0.1 ml aliquots were spread onto one non-selective agar plate: trypticase soy blood agar with 10% sheep blood (BBL, Microbiology systems, Cockeysville, USA) for aerobes. Aliquots (0.1 ml) were also spread on 5 selective agar media as follows: Beerens medium (Beerens, 1990) for *Bifidobacterium,* Rogosa agar (Imuna, Šarišské Michaľany, Slovak Republic) for *Lactobacillus,* Enterococcosel agar (BBL) for *Enterococcus*, MacConkey agar (Imuna) for *Coliforms* and Endo agar (Imuna) for *Enterobacteriaceae.* Plates for the enumeration of *aerobic* bacteria were incubated for 2 days at 37^{o}C. Colonies were counted and bacteria were Gram stained and visualized under a microscope for morphological characterization. The viable counts are expressed as the log 10 of colony forming units (cfu)g^{-1} of sample.

2.2.2 Biochemical analysis

After the collection, 1 g of digesta (jejunum, ileum, colon) was diluted in 50 ml of deionized H_2O and applied at a volume of 30 µl for analysis of SCFAs. The concentration of formic, acetoacetic, lactic, succinic, acetic, propionic, butyric and valeric acids in the intestinal content was determined by capillary isotachophoresis (ITP). The measurements were done on an „Isotachophoretic analyser ZKI 01" (SR). In the pre-separation capillary, a leading electrolyte of the following composition was used: 10^{-2} M HCl + 2.2. 10^{-2} M ε-aminocaproic acid + 0.1% methylhydroxyethylcellulosic acid, pH 4.3. As finishing electrolyte, a solution of 5.10^{-3} M caproic acid + histidine was used. This electrolytic system worked at 250µA in pre-separation and 50 µA in the analytic capillary. pH was measured by a pH meter (LP Prague, Czech Republic).

2.2.3 Disaccharidase activity

The lactase (EC 3.2.1.23), maltase (EC 3.2.1.20) and saccharase (EC 3.2.1.26) activities were measured according to Mir et al. (1997). Mucosa samples (200 mg) were homogenized for 3 min with 1 mL saline solution at 0°C. The homogenate was transferred to a test tube together with 2.5 mL (2 × aliquot) of saline solution. Three reaction tubes were filled with 100 µL of the homogenate and placed in a 37°C water bath, and then 400 µL of 56 mM lactose, maltose, saccharose in citrate buffer (pH 6.6, 0.01 mM) were added, respectively. After shaking and incubation for 30 min enzyme activity was stopped in boiling water. The reaction tubes were centrifuged at 2000 × g (30 min, 5°C). The individual enzymes were determined using enzymatic UV method (Boehringer Mannheim, Germany). Protein content in homogenate was started according to Bradford et al. (1976) and the results were expressed as µmol/ mg protein/ hour.

2.2.4 Small intestinal morphology

Fixed intestinal segments were rinsed with water, the samples were dehydrated in a graded series of absolute ethanol (30%, 50%, 70%, 90%), cleared with benzene, saturated with and embedded in paraffin. Sections of 7 µm thickness (10 slices of each sample) were stained with haematoxylin/eosin and observed under a light microscope. The length of 10 villi and depth of 10 crypts was determined by a computer operated Image C picture analysis system (Intronic GmbH, Berlin, Germany) and the IMES analysis software, using a colour video camera (Sony 3 CCD) and a light microscope (Axiolab, Carl Zeiss Jena, Germany).

2.2.5 Statistical analysis

Statistical analysis was performed using Statistic software PRIZMA (version 3.0). All the data were presented as means ± SEM. To estimate the effect of age and weaning on the concentration of SCFAs, bacterial count, disaccharidase activity and intestinal morphology, the data were evaluated statistically by one-way analysis of variance (ANOVA) followed by a multiple comparison Tukey´s test. Significant differences between the two groups of piglets were tested using analysis of variance and Student´s t-test. Probability values less than 0.05 were used as the criterion for statistical significance.

3. Results

3.1 Health status of animals

The deficit of colostral feeding on day 4 of life caused clinical symptoms of disease in 8 replacer-fed piglets (i.e. 30.8 % of the total number of 26 piglets). The other piglets from this group were healthy. The sick piglets were apathic and did not show any interest in feeding. The disease was peracute and proceeded with physiological temperature. Even though antibiotics were administered, the piglets died within 8 hrs of the first appearance of symptoms. Rectal smears and blood for hematological examination were taken from the piglets and both pathological and anatomical dissections were carried out. In the piglets, lymphocytic leukocytosis as well as hypochromic anaemia were diagnosed, and *E. coli* K88 was isolated from rectal swabs. The colonies from the final dilutions were verified by slide agglutination with K88ab antiserum (Imuna Šarišské Michaľany, Slovakia). The pathological and anatomical dissection for peracuteness of the course of the disease revealed only

petechial bleeding on seroses and mucoses of the gastrointestinal tract. Transudate in the abdominal cavity was of a deep-red colour, and the blood was uncoagulated. In the groups of suckled piglets (natural feeding) and gnotobiotic piglets the health status was good.

3.2 Acidity

The actual acidity of stomach digesta in replacer-fed piglets ranged more widely - i.e. from pH 1.7 to 3.8. During the period of observation, only on day 2 of age the pH of stomach contents of these piglets was significantly lower ($p<0.01$, $p<0.05$) than in suckled piglets with pH ranging from 2.9 to 3.7 and gnotobiotic piglets with 2.7 - 4.1 pH range (Table 1). In the proximal segment of GIT (content of duodenum and jejunum) of gnotobiotic piglets we recorded between days 7 and 28 days of age the lowest levels of pH which differed significantly on day 14 of age ($p<0.001$) in duodenum and on day 21 of age ($p<0.01$) in jejunum in comparison with replacer-fed piglets. The pH level in the caudal segment of GIT (ileal content) of suckled piglets was lower in comparison with replacer-fed piglets and significantly lower on days 2 ($p<0.05$) and 21 ($p<0.01$) of age. The ileal and colonic pH were on average lower by 0.08 to 0.5 in the group of suckled piglets and the pH values ranged from 6.27 to 7.17 and from 6.50 to 7.21 compared to replacer-fed piglets in which the pH of the ileal content ranged from 6.93 to 7.64 and the pH of the colonic content ranged from 6.24 to 7.53.

3.3 Effect of age and weaning on production of SCFAs in the intestinal tract of gnotobiotic and conventionally bred piglets

3.3.1 Conventional suckled piglets (natural feeding)

Concentration of both acetoacetic and acetic acid in the jejunal content of suckled piglets (Table 2) within the period of milk nutrition was the highest at 7 days of age ($p < 0.001$ and $p < 0.01$, respectively). Subsequently the values declined at 2 weeks of age to 14.76 mmol/l of acetoacetic and to 28.02 mmol/l of acetic acid. This decline continued in acetoacetic acid by day 35 of age to 6.10 mmol/l and in acetic acid by week 4 of life to 7.71 mmol/l ($p < 0.01$). The concentration of lactic acid in jejunal contents was comparable to that of both acetoacetic and acetic acid, with a mean decline of 11.85 mmol lactic acid/l between day 2 and day 21. But a pronounced increase in the concentration of lactic acid was recorded at 1st week post-weaning - i.e. 53.91 mmol/l. The course of the concentration of both acetoacetic and lactic acid in the ileal content (Table 3) was largely similar to that recorded in the jejunal content. Under the influence of more diverse populations of microorganisms, the conditions in the colonic content changed. Propionic acid concentration (Table 4) increased gradually up to weaning (28 days) and then markedly after weaning (day 35: $p < 0.01$ and on day 42: $p < 0.05$). The most pronounced production of acids in the colonic content was observed in acetoacetic acid with highest concentrations at day 14 and 28 of age ($p < 0.001$ and $p < 0.001$) and a sudden 4-fold decline at 1st week post-weaning ($p < 0.01$). In acetic acid, a gradual increase in values was recorded from 7 days of age (11.91 mmol/l), with its highest concentration at 2 weeks post-weaning ($p < 0.001$).

3.3.2 Conventional replacer-fed piglets (artificial feeding)

In both acetoacetic and lactic acid, the highest levels in the jejunal content in replacer-fed piglets (Table 2) were recorded at 7 days of age (19.89 mmol/l and 24.92 mmol/l, $p < 0.01$,

	Segments of GIT					SEM	p - value	
	St	D	J	Ile	C		SC × RFP	GP × RFP
Day 2								
Suckled piglets	3.7	5.5	6.1	6.6a	7.2	0.27	$p < 0.05$	-
Replacer-fed piglets	1.7ba	5.7	6.8	7.4	7.4	0.12	$p < 0.01$	$p < 0.05$
Gnotobiotic piglets	4.1	ND	7.2	8.0	7.7	0.39	-	-
Day 7								
Suckled piglets	2.9	5.6	6.2	6.3	6.5	0.09	NS	-
Replacer-fed piglets	3.8	5.8	6.0	6.9	6.3b	0.22	NS	$p < 0.01$
Gnotobiotic piglets	4.1	4.5	5.9	7.4	7.0	0.56	-	-
Day 14								
Suckled piglets	3.4	5.4	6.1	7.2	6.7	0.09	NS	-
Replacer-fed piglets	2.5	5.5	5.9	7.1	7.5	0.18	NS	-
Gnotobiotic piglets	3.5	4.2c	5.4	7.0	6.4	0.23	-	$p < 0.001$
Day 21							$p < 0.05$	
Suckled piglets	3.3	5.2	6.2a	6.8b	7.4	0.15	$p < 0.01$	-
Replacer-fed piglets	3.4	5.4	6.7	7.5	7.5	0.23	-	-
Gnotobiotic piglets	3.1	4.2	5.9b	6.9	6.7	0.34	-	$p < 0.01$
Day 28								
Suckled piglets	3.2	5.8	5.9	6.6	6.5	0.18	NS	-
Replacer-fed piglets	2.9	6.0	6.7	7.6	6.2	0.25	NS	NS
Gnotobiotic piglets	2.7	4.4	5.8	6.7	6.6	0.34	-	NS

ND- not detectable, NS- not significant, SP- suckled piglets, GP- gnotobiotic piglets, RFP- replacer-fed piglets, St- stomach, D- duodenum, J- jejunum, Ile- ileum, C- colon, GIT- gastrointestinal tract
Significantly different (SP,GP vs RFP): a ($p < 0.05$), b ($p < 0.01$), c ($p < 0.001$)

Table 1. The pH along the gastrointestinal tract of gnotobiotic and conventionally bred piglets

respectively), with a slight decline in the concentration up to the end of observation. Concentrations of acetoacetic acid in the colonic content (Table 4) were similar to those in the jejunal content except on day 7 of age when a value of 24.09 mmol/l was recorded ($p < 0.05$). With lactic acid, the highest concentration was seen on day 7 of age (31.17 mmol/l), with a sudden 10-fold decline from day 14 of age (3.15 mmol/l) up to 28 days of life. Acetic acid concentration was relatively stable from 2 to 21 days of life. Thereafter, a marked increase in the concentration was observed at 4 weeks of life ($p < 0.01$).

3.3.3 Gnotobiotic piglets

The concentration of acetoacetic acid in the jejunal content of gnotobiotic piglets (Table 2) reached the highest level at the age of 7 days, in the period of milk nutrition ($p < 0.05$), in comparison with the concentration recorded three hours after birth (3.35 mmol/l). A more

	Age (days)									p - value					
	0	2	7	14	21	28	35	42	SEM	0 × 7	2 × 7	7 × 28	28 × 35	SC × RFP	GP × RFP
Formic acid															
Suckled piglets	ND	4.86	5.09	5.77	7.82	8.49	11.74	8.49	1.37	NS	NS	NS	NS	NS	-
Replacer-fed piglets	ND	7.00a	10.85a	11.50	7.21a	ND	ND	ND	1.82	NS	NS	-	-	p < 0.05	p < 0.05
Gnotobiotic piglets	1.00	6.64	7.54	4.46	3.62	10.67	4.58	ND	1.20	NS	NS	NS	NS	-	NS
Acetoacetic acid															
Suckled piglets	ND	18.12	28.69***	14.76	9.11	10.51	6.10	13.51	2.96	NS	p< 0.001	NS	NS	NS	-
Replacer-fed piglets	ND	9.11a	19.89	9.05	12.24	ND	ND	ND	1.81	NS	NS	-	-	NS	p < 0.05
Gnotobiotic piglets	3.35	6.47	17.78*	17.70	12.93	13.26	8.02*	ND	2.40	p < 0.05	NS	NS	p < 0.05	-	NS
Lactic acid															
Suckled piglets	ND	22.98	27.52*	18.18	11.13	17.89	53.91	30.26	8.17	NS	p < 0.05	NS	NS	NS	-
Replacer-fed piglets	ND	17.61	24.92**	23.75	21.98b	ND	ND	ND	1.10	NS	p < 0.01	-	-	p < 0.01	NS
Gnotobiotic piglets	14.22	16.57	23.59	19.96	21.94	21.38	31.25	ND	3.22	NS	NS	NS	NS	-	NS
Succinic acid															
Suckled piglets	ND	9.05	15.10	11.74c	5.36	4.24	5.92	11.74	1.77	NS	NS	NS	NS	p < 0.001	-
Replacer-fed piglets	ND	2.68	6.44	4.59	6.87	ND	ND	ND	0.89	NS	NS	-	-	NS	NS
Gnotobiotic piglets	2.01	2.01	2.67	4.09	5.79	6.03	4.40	ND	0.66	NS	NS	NS	NS	-	NS
Acetic acid															
Suckled piglets	8.50	21.14a	33.05**	28.02c	10.17	7.71**	11.45	11.69	2.99	NS	p < 0.01	p < 0.01	NS	p< 0.05, p< 0.001	-
Replacer-fed piglets	ND	6.71	21.43	13.49a	14.08	ND	ND	ND	2.49	NS	NS	-	-	NS	p < 0.05
Gnotobiotic piglets	6.57	5.20	8.18	5.80	12.86	22.11**	15.95	ND	1.75	NS	NS	NS	(0×28) p < 0.01	-	NS

Table 2. Effect of age, weaning and diets on production of SCFAs in the jejunum (mmol/l) of gnotobiotic and conventionally bred piglets. ND- not detectable, NS- not significant, SP- suckled piglets, GP- gnotobiotic piglets, RFP- replacer-fed piglets. Significantly different (SC,GP vs RFP): a ($p < 0.05$), b ($p < 0.01$), c ($p < 0.001$)

pronounced post-weaning decrease in the level of acetoacetic acid was recorded one week after weaning ($p < 0.05$). The proportion of lactic acid was relatively stable from day 7 to 28 of life and ranged between 19.96 and 23.59 mmol/l. Afterwards, in the 5th week of life, the lactic acid level increased to 31.25 mmol/l. Similarly, acetic acid level was relatively stable during the first two weeks of life of piglets, then increased significantly ($p < 0.01$) at the age of 28 days compared to the level at 3 hours after birth (6.57 mmol/l). The most important production of acids in the colon of gnotobiotic piglets (Table 4) was the production of acetic acid which remained relatively stable between days 7 and 35 of life and the difference between its concentration recorded at 3 hours after the birth (6.94 mmol/l) and that determined on day 7 of life (41.98 mmol/l) was significant ($p < 0.05$). A significant difference in concentration of lactic acid was recorded on day 28 of age ($p < 0.001$, $p < 0.01$) compared to the level on the second day (8.18 mmol/l) and 21st day (14.49 mmol/l). Subsequently, we recorded a non-significant, 3-fold increase in the level of this acid at 5 weeks of age (to 76.30 mmol/l). A similar tendency was recorded for acetoacetic and propionic acid in the colonal content with the exception of the level recorded at 3 hours after the birth. The level of acetoacetic acid was significantly higher on day 21 of age (16.22 mmol/l, $p < 0.01$) compared to the second day of life (6.71 mmol/l).

3.4 Effect of diets on production of SCFAs in the intestinal tract of gnotobiotic and conventionally bred piglets

The concentration of acetoacetic acid in the jejunal content (Table 2) was higher in suckled piglets at 7 days of life (28.69 mmol/l) compared to the replacer-fed animals in which the level of the above acid represented 19.89 mmol/l. In both groups of piglets observed, well-balanced levels of the above acid were recorded thereafter. The course of the concentration of lactic acid up to day 7 of age was the same in 3 groups of piglets with a subsequent increase in replacer-fed piglets at 21 days of age ($p < 0.01$) compared to suckled animals. In the acetic acid of suckled piglets, significantly higher levels were recorded at day 2 and 14 of age compared to the replacer-fed piglets ($p < 0.05$, $p < 0.001$). The proportion of acetic acid in gnotobiotic piglets was up to two weeks of life considerably lower in comparison with both investigated groups and the decrease on day 14 of life was significant ($p < 0.05$) in comparison with replacer-fed piglets. The course of the concentration of acetoacetic acid in the ileal content (Table 3) was the same in all 3 observed groups except at 3 weeks of age of replacer-fed piglets. While the content of acetoacetic acid represented 17.11 mmol/l in suckled piglets at 3 weeks of life, in the non-colostral group a decline to 8.78 mmol/l was observed. The level of acetic acid in the ileal contents of suckled piglets was higher throughout the period of investigation ranging from about 7 to 21.5 mmol/l compared with the replacer-fed piglets which showed the highest concentration on day 2 ($p < 0.001$) and 14 of life ($p < 0.05$). Insignificantly higher concentrations of acetic acid in the ileal content were observed also in gnotobiotic piglets (2.05 - 13.69 mmol/l) compared to replacer-fed piglets. A similar tendency was recorded in lactic acid, in the ileal content up to 14 days of age, with higher concentrations in suckled piglets as compared to the replacer-fed piglets in which the values later ranged about 8.2 till 21.4 mmol/l and a significantly higher level of the acid was recorded at 7 days of age ($p < 0.001$). Higher production of lactic acid by gnotobiotic piglets (0.6 - 10.14 mmol/l) was observed throughout the observation period in comparison with replacer-fed piglets with a significant increase on day 7 of age ($p < 0.01$).

	Age (days)									p - value		
	0	2	7	14	21	28	35	42	SEM	28 × 35	SC × RFP	GP × RFP
Formic acid												
Suckled piglets	ND	6.37	6.70	4.79	9.23	14.65	12.19	13.08	1.50	NS	NS	-
Replacer-fed piglets	ND	9.66	8.38	14.35a	8.55	7.41	ND	ND	1.20	-	$p < 0.05$	NS
Gnotobiotic piglets	3.28	9.02	26.17	22.82	20.84a	12.68	21.47	ND	3.57	NS	-	$p < 0.05$
Acetoacetic acid												
Suckled piglets	ND	9.06	26.17	13.58	17.11	14.16	4.18*	5.14	3.48	$p < 0.05$	NS	-
Replacer-fed piglets	ND	5.39	22.48	10.12a	8.78	9.13	ND	ND	2.75	-	NS	$p < 0.05$
Gnotobiotic piglets	6.37	6.84	13.42	6.14	13.08	7.68	6.17	ND	2.00	NS	-	NS
Lactic acid												
Suckled piglets	ND	25.63	26.91c	21.64	15.52	37.58	60.11	55.90	12.61	NS	$p < 0.001$	-
Replacer-fed piglets	ND	6.27	14.42	13.41	20.30	16.17	ND	ND	1.79	-	NS	NS
Gnotobiotic piglets	8.72	6.87	24.56b	12.25	24.40	20.67	23.73	ND	7.34	NS	-	$p < 0.01$
Succinic acid												
Suckled piglets	ND	7.24	23.62	11.07c	11.07	9.72	4.85	5.47	1.97	NS	$p < 0.001$	-
Replacer-fed piglets	ND	4.72	2.91	3.40	8.38	7.51	ND	ND	0.85	-	NS	NS
Gnotobiotic piglets	1.87	2.91	3.69	4.56	5.02	5.03	2.50	ND	0.79	NS	-	NS
Acetic acid												
Suckled piglets	ND	35.23c	21.81	27.18a	25.00	34.00	11.34	15.83	5.74	NS	$p < 0.05$, $p < 0.001$	-
Replacer-fed piglets	ND	15.10	10.47	20.13	19.86	13.43	ND	ND	2.03	-	NS	NS
Gnotobiotic piglets	7.71	11.14	24.16	22.18	32.08	21.58	25.41	ND	5.48	NS	-	NS
Propionic acid												
Suckled piglets	ND	ND	3.18	6.71	8.38	ND	ND	ND	1.06	-	NS	-
Replacer-fed piglets	ND	ND	5.30	4.57	5.03	7.24	ND	ND	1.36	-	NS	NS
Gnotobiotic piglets	ND	2.21	6.17	ND	1.84	3.52	1.17	ND	0.48	NS	-	NS
Butyric acid												
Suckled piglets	ND	2.68	ND	ND	1.67	ND	2.25	3.48	0.68	-	NS	-
Replacer-fed piglets	ND	2.51	ND	ND	ND	3.02	ND	ND	0.84	-	NS	NS
Gnotobiotic piglets	ND	2.01	ND	3.52	3.38	7.24	1.71	ND	1.03	NS	-	NS

Table 3. Effect of age, weaning and diets on production of SCFAs in the ileum (mmol/l) of gnotobiotic and conventionally bred piglets. ND - not detectable, NS- not significant, SP- suckled piglets, GP- gnotobiotic piglets, RFP- replacer-fed piglets. Significantly different (SP,GP vs RFP): a ($p < 0.05$), b ($p < 0.01$), c ($p < 0.001$)

The value of acetoacetic acid in the colonic content (Table 4) was significantly higher in the group of suckled piglets from day 14 to 28 of age ($p < 0.01$), compared to replacer-fed

	Age (days)									p - value						
	0	2	7	14	21	28	35	42	SEM	2 × 7	7 × 14	21 × 28	28 × 35	28 × 42	SC × RFP	GP × RFP
Formic acid																
Suckled piglets	9.22	3.85	4.12	5.03	6.59	6.04	9.29	8.04	0.67	NS	NS	NS	NS	NS	NS	-
Replacer-fed piglets	5.36	7.21b	10.77c	6.94	6.20b	5.36	ND	ND	0.83	NS	NS	NS	NS	-	p < 0.01, p < 0.001	p < 0.01
Gnotobiotic piglets	3.08	9.73	5.56	5.90	2.76	2.48	2.01	ND	1.43	NS	NS	NS	NS	-	-	NS
Acetoacetic acid																
Suckled piglets	3.18	7.55	12.91	71.3b***	54.58b	67.2b***	16.95**	18.00	4.39	NS	p<0.001	NS	p < 0.01	p<0.001	p < 0.01	-
Replacer-fed piglets	3.55	11.50a	24.09*	12.41	8.58	8.69	ND	ND	1.37	p < 0.05	NS	NS	-	-	p < 0.05	NS
Gnotobiotic piglets	9.09	6.71	10.57	22.85	16.22c**	18.29	12.84	ND	3.12	NS	NS	NS	p < 0.01 (2×21)		-	p < 0.001
Lactic acid																
Suckled piglets	5.09	5.19	3.01	3.23	2.23	3.35	4.36	4.02	0.42	NS	NS	NS	NS	NS	NS	-
Replacer-fed piglets	6.94	4.96	31.17	3.15	4.19	4.69	ND	ND	2.96	NS	NS	NS	-	-	NS	NS
Gnotobiotic piglets	21.61	8.18a	19.09	19.39b	14.49b	26.34b ** ***	76.3	ND	6.42	NS	NS	p < 0.01	p < 0.001 (2×28)		-	p<0.05, p<0.01
Acetic acid																
Suckled piglets	27.17b	15.17	11.91	46.81***	57.22	71.14	89.99*	95.3 ***	2.39	NS	p<0.001	NS	p < 0.05	p<0.001	p < 0.01	-
Replacer-fed piglets	11.47	44.86bb	46.03	46.06	49.66	64.13a**	ND	ND	6.95	NS	NS	p < 0.01	NS	NS	p < 0.01	p<0.01, p<0.05
Gnotobiotic piglets	6.94	10.06	41.98*	44.96	44.20**	44.90	46.95	ND	8.05	p < 0.05	NS	NS	NS		-	NS
Propionic acid																
Suckled piglets	8.72	ND	ND	16.27	13.08	22.59	59.63**	71.14*	4.46	NS	NS	NS	p < 0.01	p < 0.05	NS	-
Replacer-fed piglets	6.37	ND	11.07	14.76	18.28b	21.81b	ND	ND	4.18	NS	NS	NS	-	-	NS	p < 0.01
Gnotobiotic piglets	1.34	4.69	5.02	9.73	5.52	7.37	6.54	ND	1.28	NS	NS	NS	NS	-	-	NS
Butyric acid																
Suckled piglets	ND	3.39	2.01	11.74	14.76	14.43	11.40	14.65	3.74	NS	NS	NS	NS	NS	-	-
Replacer-fed piglets	2.55	11.12a	17.11	14.93	3.35	3.35	ND	ND	2.25	NS	NS	NS	-	-	p < 0.05	NS
Gnotobiotic piglets	3.02	ND	3.01	8.21	6.28	3.35	2.14	ND	1.27	-	NS	NS	NS	-	-	NS
Valeric acid																
Suckled piglets	ND	ND	ND	ND	5.53	3.91	6.71	5.20	0.68	-	-	NS	NS	NS	NS	-
Replacer-fed piglets	ND	5.03	2.01	2.14	ND	ND	ND	ND	0.60	NS	NS	-	-	-	NS	-
Gnotobiotic piglets	ND	ND	ND	ND	ND	5.26	ND	2.93	1.57	-	-	-	-	-	-	-

Table 4. Effect of age, weaning and diets on production of SCFAs in the colon (mmol/l) of gnotobiotic and conventionally bred piglets. ND- not detectable, NS- not significant, SP- suckled piglets, GP- gnotobiotic piglets, RFP- replacer-fed piglets. Significantly different (SP,GP vs RFP): a ($p < 0.05$), b ($p < 0.01$), c ($p < 0.001$)

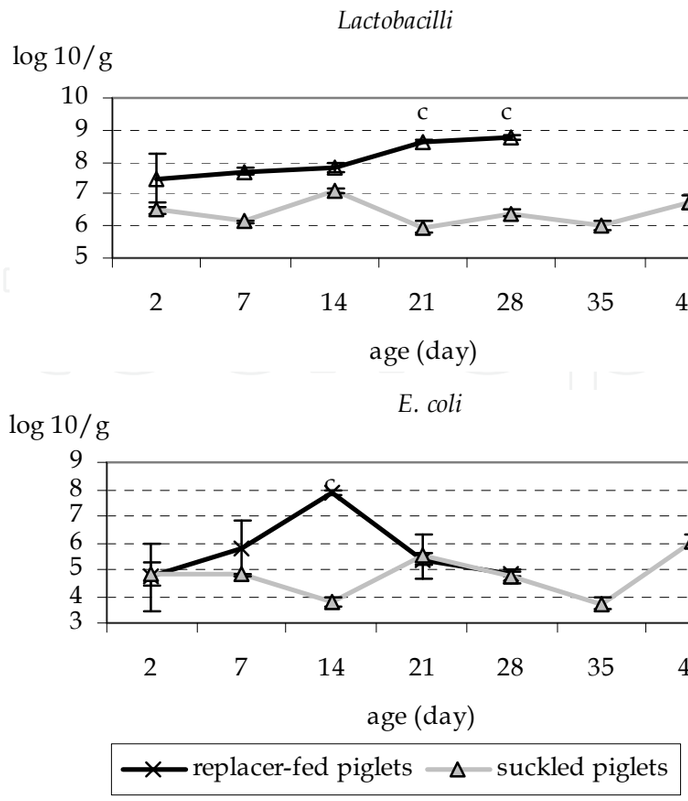
piglets. In the period between weeks 2 to 4 of life, production of this acid by gnotobiotic piglets was also higher in comparison with replacer-fed piglets with significant increase at 3 weeks of life ($p < 0.001$). While the dynamics of lactic acid in the colon of suckled and replacer-fed piglets was similar with the exception of week 1, and its levels were low and did not exceed 7 mmol/l, gnotobiotic piglets produced higher level of this acid with peaks at 2 days ($p < 0.05$) and 14, 21 and 28 days of age ($p < 0.01$) compared to the replacer-fed animals. Dynamics of acetic acid in colonal content of suckled piglets resembled that in jejunal and ileal contents and reached higher levels with the exception of days 2 and 7 of age. Significant difference ($p < 0.01$) was observed at 3 hours after the birth in comparison with replacer-fed piglets. Acetic acid in replacer-fed piglets showed an opposite trend in the proximal section of the intestinal tract where we recorded a gradual increase in concentrations up to the end of observation with highest levels at 2 days ($p < 0.01$) and 28 days of age ($p < 0.05$) compared to the gnotobiotic piglets. In the colon of replacer-fed piglets we recorded also higher production of propionic acid on days 21 and 28 of age ($p < 0.01$) compared to the gnotobiotic piglets and butyric acid at 2 days of age ($p < 0.05$) compared to suckled piglets.

3.5 Effect of diets on development of microflora in the digestive tract of suckling piglets and replacer-fed piglets

In suckled piglets, an increase in the followed microflora population was recorded towards the caudal part of the intestine. Bacterial populations ranged from 4 to 8 log cfu/g in the jejunum, from 4 to 9 log cfu/g in the ileum, and from 6 to 9 log cfu/g in the caecum. The lactobacilli in the ileum slightly increased within the period of observation ranging from 1 to 2 log. The cfu of *E. coli* and *Enterobacteriaceae* in the ileum remained more or less stable over time, while they declined in the caecum. The course of development of total aerobes was similar in the jejunum and ileum throughout the period of observation, in the colon, however, total aerobes populations maintained at a constant level of 9 log cfu/g.

Likewise, in conventional piglets fed on milk replacement, an increase in the microflora population was observed towards the caudal part of the intestine. Bacterial populations ranged from 4 to 8 log cfu/g in the jejunum, from 4 to 9 log cfu/g in the ileum and from 6 to 9 log cfu/g in the caecum. In all parts of the intestine, *E. coli* and *Enterobacteriaceae* increased by 1 to 3 log units between days 2 and 14, and decreased thereafter until day 28. In the jejunum and ileum lactobacilli and enterococci slightly increased throughout the period of observation, in the colon, however, lactobacilli populations persisted at a constant level of 9 log cfu/g. *Enterococcus* spp. in colon contents declined by about 2 log units until 28 days of age. The course of the development of total aerobes was the same throughout the period of observation in all parts of the intestine.

Total lactobacilli populations in the jejunal content were significantly higher in the group of conventional replacer-fed piglets with highest counts on days 21 and 28 of age ($p < 0.001$ and $p < 0.001$) compared to the group of suckled piglets (Figure 1). The course of *E. coli* development in the jejunal content was the same in both observed groups except at 2 weeks of age. Whereas total *E. coli* populations were lower in the group of suckled piglets at 2 weeks of life, i.e. 3.78 log cfu/g, a significant increase was observed in group of replacer-fed piglets ($p < 0.001$). In conventional piglets fed on milk replacement, an increase in enterococci populations (Table 5) in the jejunal content was seen throughout the period of



Each bar represents the mean ± SE of 3 piglets.
Significantly different (SP vs RFP): c ($p < 0.001$)

Fig. 1. Effect of diets on bacterial population (lactobacilli and *E.coli*) in the jejunum of suckling piglets and replacer-fed piglets

observation. In suckled piglets, on the other hand, total enterococci populations were by 0.5 - 2.5 log lower. The highest enterococci populations in the jejunal content of replacer-fed piglets were recorded at 21 and 28 d of age ($p < 0.001$ and $p < 0.001$, respectively). Total counts of *Enterobacteriaceae* in the jejunal content were the highest in replacer-fed piglets at 2 (6.95 log cfu/g) and 14 days of age ($p < 0.001$) compared to the group of suckled piglets in which lower numbers (by 3 logs) were seen at 2 days of life (4.00 log cfu/g) and at 14 days of age the populations were by 3.6 log lower (4.11 log cfu/g). The course of the development of total aerobes in the jejunal content was the same in both groups of piglets, however, with the difference that in replacer-fed piglets, the total numbers of observed bacteria were by 0.5 to 1.2 log higher with significantly higher numbers at 7 ($p < 0.001$), 14 ($p < 0.05$), and 21 days of age ($p < 0.001$).

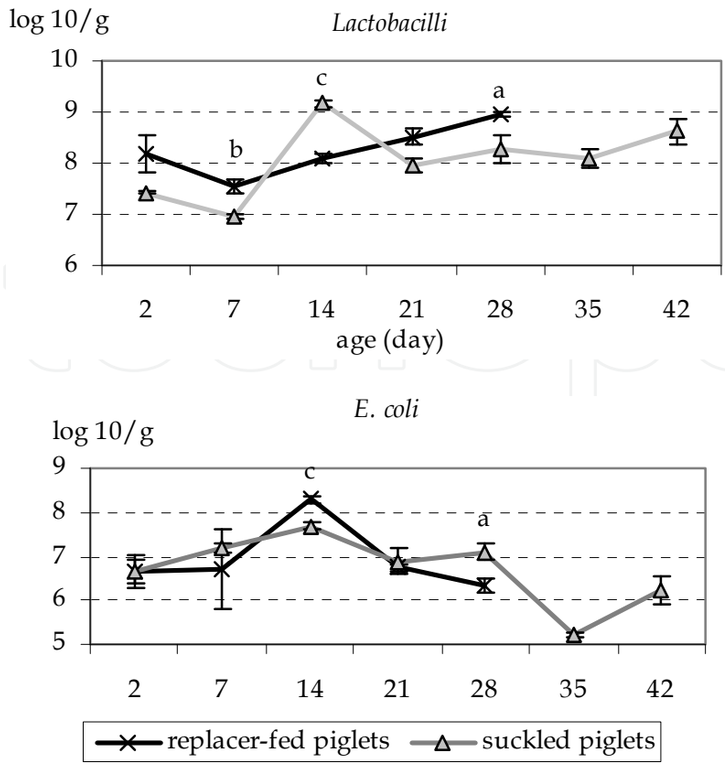
In the ileal content of replacer-fed piglets we detected higher lactobacilli populations (Figure 2) compared to the suckled animals throughout the period of observation except at 14 days of age with significantly higher numbers at 7 and 21 days of age ($p < 0.01$ and $p < 0.05$). The course of development of *E. coli* was the same in both groups, however, with the difference that in the group of suckled piglets the total numbers were higher by 0.5 log except at 14 days of age when an increase in the total *E. coli* populations was recorded ($p < 0.001$). Total enterococci populations in the ileal content (Table 5) were higher by 1 to 3.5 log in replacer-

	Content						
	Jejunum		Ileum		Caecum		
	SP	RFP	SP	RFP	SP	RFP	SEM
Day 2							
Enterococci	6.36	6.83	6.10	7.70***	8.08	8.69*	0.31
Enterobacteiaceae	4.00	6.95	6.89***	4.55	8.95	7.92	0.25
Total aerobes	7.35	7.97	9.04	8.31	9.33	9.35	0.19
Day 7							
Enterococci	6.19	7.22	3.96	7.42***	7.49	8.73*	0.29
Enterobacteiaceae	4.43	4.02	7.24***	5.27	8.51	8.35	0.19
Total aerobes	6.56	8.15***	7.76	8.45**	9.24	9.57	0.14
Day 14							
Enterococci	5.12	6.80	5.06	7.49***	6.82	7.41	0.31
Enterobacteiaceae	4.11	7.72***	7.16***	8.17	7.37	9.42	0.26
Total aerobes	7.51	8.13*	7.46	8.83**	9.15	9.43	0.19
Day 21							
Enterococci	4.93	7.42***	6.01	6.93***	6.91	6.57	0.14
Enterobacteiaceae	5.38	4.69	6.69	6.57	7.10	8.13	0.19
Total aerobes	6.93	8.22***	8.77	9.35	9.13	9.12	0.29
Day 28							
Enterococci	5.52	7.46***	6.38	8.95***	7.17	6.94	0.16
Enterobacteiaceae	4.81	4.74	6.71**	5.96	6.07	6.53	0.28
Total aerobes	7.95	7.65	8.89	9.20	9.05	8.79	0.21
Day 35							
Enterococci	4.45	ND	5.15	ND	7.17	ND	0.39
Enterobacteiaceae	3.00	ND	5.79	ND	5.98	ND	0.19
Total aerobes	6.61	ND	9.26	ND	9.38	ND	0.14
Day 42							
Enterococci	6.75	ND	7.94	ND	8.91	ND	0.19
Enterobacteiaceae	5.38	ND	5.30	ND	5.36	ND	0.14
Total aerobes	7.80	ND	8.07	ND	9.13	ND	0.51

SP- suckled piglets (n=3), RFP- replacer-fed piglets (n=3), ND- not detectable
*p < 0.05, **p < 0.01, ***p < 0.001

Table 5. Effect of diets on bacterial population (log_{10} cfu/g of digesta) at various locations along the intestinal tract of suckling piglets and replacer-fed piglets

fed piglets than those in suckled piglets throughout the period of observation with significant difference between days 7 and 28 of age ($p < 0.001$). In suckled piglets, total *Enterobacteriaceae* populations were by 2 log higher by 1 week of life compared to the

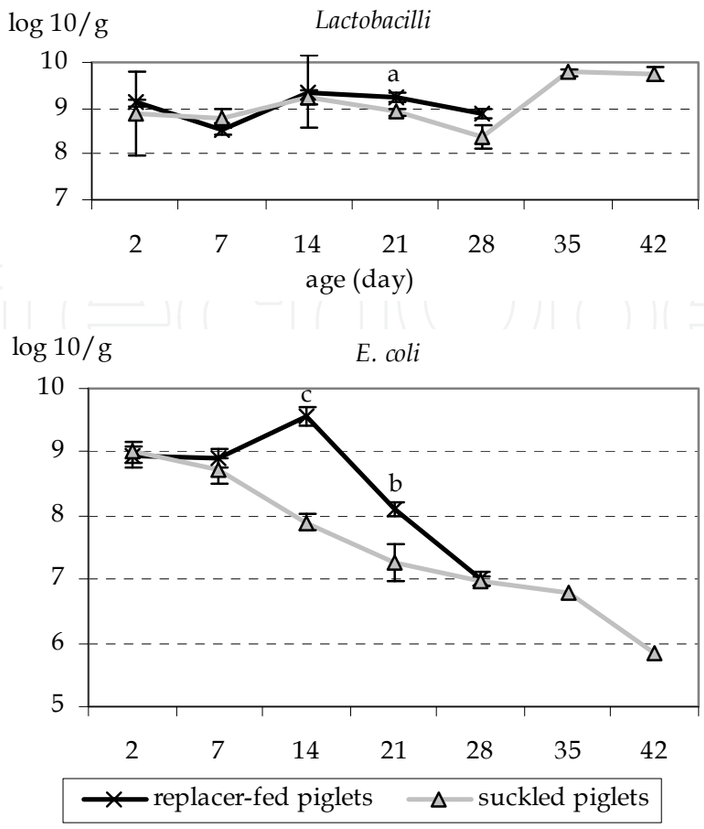


Each bar represents the mean ± SE of 3 piglets
Significantly different (SP vs RFP): a ($p < 0.05$), b ($p < 0.01$), c ($p < 0.001$)

Fig. 2. Effect of diets on bacterial population (lactobacilli and *E.coli*) in the ileum of suckling piglets and replacer-fed piglets

replacer-fed piglets. Thereafter, the populations of observed bacteria slightly declined up to the end of the period of observation. A significant increase in the *Enterobacteriaceae* populations in replacer-fed piglets was recorded at 14 days of age ($p < 0.001$). Numbers of total aerobes in the ileal content were by 0.5 - 1.5 log higher in replacer-fed piglets compared to colostral animals with significant difference on day 7 ($p < 0.01$) and 14 of age ($p < 0.01$).

In the caecum of suckled piglets, *E. coli* populations gradually declined throughout the period of observation (Figure 3) compared with replacer-fed piglets in which a significant increase in numbers was recorded at 14 and 21 days of age ($p < 0.001$ and $p < 0.01$, respectively). The course of the development of lactobacilli and total aerobes in the content of the caecum was the same. Total enterococci populations in replacer-fed piglets (Table 5) were by 0.6 - 1.2 log higher by day 14 of age, with significant enterococci populations at 2 and 7 days of age ($p < 0.05$ and $p< 0.05$). After day 14, the cfu of *Enterococcus* spp. in the caecum contents of replacer-fed piglets were about 0.3 log units lower than those of suckled piglets. A significant increase in the *Enterobacteriaceae* populations in replacer-fed piglets compared to the second group was recorded from day 14 of age ($p < 0.001$) to the end of the period of observation ($p < 0.01$) at 21 days of age and ($p < 0.05$) at 28 days of age.



Each bar represents the mean ± SE of 3 piglets.
Significantly different (SP vs RFP): a ($p < 0.05$), b ($p < 0.01$), c ($p < 0.001$)

Fig. 3. Effect of diets on bacterial population (lactobacilli and *E.coli*) in the colon of suckling piglets and replacer-fed piglets

3.6 Effect of age, weaning and diets on development of intestinal morphology in gnotobiotic and conventionally bred piglets

From day 2 of age we recorded a gradual increase in body weight of gnotobiotic piglets from 0.75 kg up to 1.80 kg ($p < 0.05$) on day 14 of age and 3.90 kg ($p < 0.01$) on day 21 of age. On day 28 (day of weaning) and one week after weaning the body weight of piglets was decreased insignificantly (Table 6). The increase in relative weight of the small intestine resembled that of the large intestine throughout the period of investigation with the exception that the relative weight of the large intestine in comparison with the weight on day 2 of age was increased significantly on day 21 ($p < 0.05$), 28 ($p < 0.05$) and 35 of age ($p < 0.001$). Similar trend of body weight increase as that recorded in gnotobiotic piglets was observed also in conventional piglets, increasing from 1.41 kg at 2 days of age to 3.50 kg at 14 days of age ($p < 0.05$). The body weight of piglets increased gradually up to the end of observation with significant increase on day 35 of life ($p < 0.01$) in comparison with the day of weaning (Table 6).

The development of relative weight of the small and large intestine of suckled piglets was similar and the relative weights decreased gradually between days 2 and 21 of age. On day

	Age (day)						
	2	7	14	21	28	35	SEM
Suckled piglets							
Weight (kg)	1.41	2.05	3.50*a	4.30	5.35	8.55**a	0.39
Small intestinal weight (g/kg)	67.88	64.59	43.59	41.88	44.27	156.6***a	7.08
Large intestinal weight (g/kg)	25.52	24.65	15.68	15.53	23.38	125.4***a	2.38
Gnotobiotic piglets							
Weight (kg)	0.75	1.29	1.80*	3.90**	3.65	3.25	0.46
Small intestinal weight (g/kg)	42.47	52.59	53.02	64.68b	55.41	60.67	6.14
Large intestinal weight (g/kg)	13.89	18.31	20.07	47.41*b	42.1*	85.63***	4.07

Significantly different (SP vs GP): a ($p < 0.05$), b ($p < 0.01$)
*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

Table 6. Effect of age, weaning and diets on weight, gut weight of suckling piglets and gnotobiotic piglets

28 of age the relative weights showed an insignificant increase but in the first week post-weaning the relative weights of both small and large intestine increased significantly ($p < 0.001$) in comparison with all periods of investigation. The weight of conventional piglets was greater throughout the experiment and significant differences ($p < 0.05$) were recorded on days 14 and 35. Completely opposite trend was recorded for relative weights of small and large intestines of these piglets (Table 6). While relative weights of intestines of gnotobiotic piglets gradually increased throughout the experiment with the exception of day 28 of life, the weight of small and large intestine in suckled piglets decreased gradually between days 2 and 21 of life. Between days 2 and 28 of life the relative weight of the intestines of gnotobiotic piglets was higher compared to conventional piglets with significant difference on day 21 of life ($p < 0.01$). In the post-weaning period (one week post-weaning), the weight of small and large intestine of suckled piglets was significantly higher ($p < 0.05$) in comparison with gnotobiotic piglets in the same period of observation.

The development of the height of villi in individual segments of the small intestine of gnotobiotic piglets is shown in Table 7. On day 21, the height of villi in the duodenum was decreased significantly ($p < 0.01$). A decrease in their length was also recorded on day 28 but the difference was insignificant. In the duodenum of suckled piglets, the height of villi (Table 7) increased significantly ($p < 0.001$) in the period between birth and day 14 of age. Subsequently, their length decreased gradually up to the weaning ($p < 0.01$). Throughout the experiment, the willi in the duodenum of gnotobiotic piglets were higher and their height differed significantly from that of conventional piglets at 3 hours after birth, days 2 and 7 of age ($p < 0.01$) and day 14 of age ($p < 0.05$) (Figure 4).

The length of jejunal villi of gnotobiotic piglets (Table 7) increased from day 0 up to day 2 of age reaching maximum of 725.18 μm. In the subsequent 5 weeks, the length of villi decreased gradually down to 387.44 μm in the week after weaning and the decrease was significant on days 14 ($p < 0.05$) and 21 of age ($p < 0.001$). In the jejunum of suckled piglets we observed a gradual but insignificant increase in villi height in the period from birth up to day 7 of life. In the following period, from day 14 of age till one week post-weaning we recorded gradual decrease in the height of villi, significant on days 21 ($p < 0.001$) and 35 of

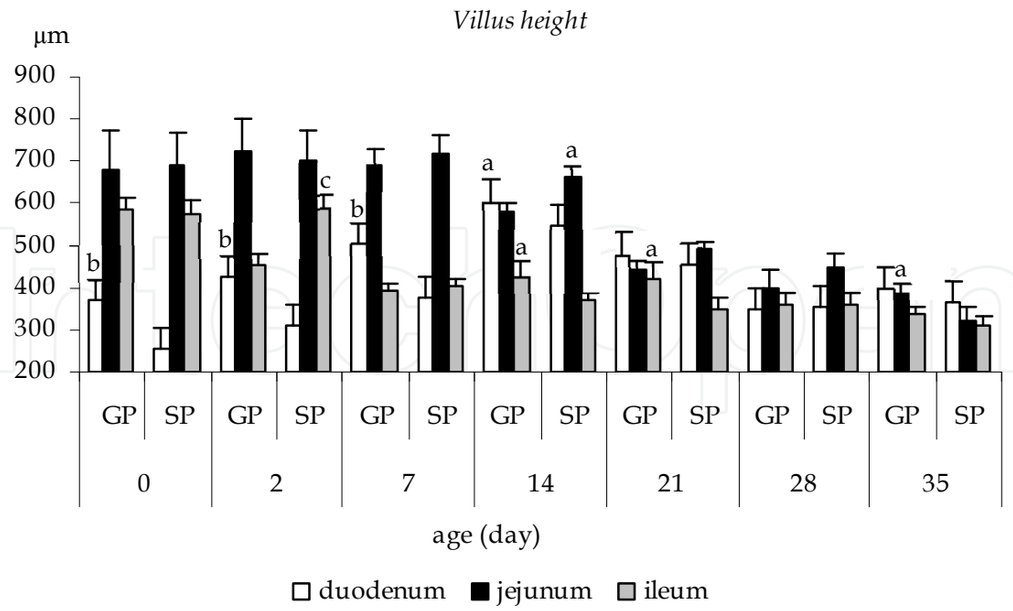
	Content						
	Duodenum		Jejunum		Ileum		
	SP	GP	SP	GP	SP	GB	SEM
Day 0							
Villus height, µm	253.67	371.29	689.68	679.43	574.80	587.37	47.11
Crypt depth, µm	129.13	81.63	53.67	67.12	48.14	79.43	1.96
Day 2							
Villus height, µm	308.42***	424.81	701.57	725.18	588.29	455.05	45.66
Crypt depth, µm	140.23	104.15	69.77	72.66	77.77	82.54	2.73
Day 7							
Villus height, µm	374.98***	502.63	718.21	691.74	401.54***	393.00	28.60
Crypt depth, µm	188.00	124.10	83.82	90.97	71.18	111.35	5.03
Day 14							
Villus height, µm	547.83***	605.14	661.59	581.23*	369.52	424.16	29.03
Crypt depth, µm	151.79	119.27	92.53	103.47	178.96	118.22	3.96
Day 21							
Villus height, µm	454.48**	478.43**	492.42***	442.86***	351.01	423.29	34.21
Crypt depth, µm	168.54	162.33*	129.44	136.17	134.26	176.15*	3.60
Day 28							
Villus height, µm	351.81**	349.21	447.67	401.93	361.69	358.57	31.64
Crypt depth, µm	220.94	197.82	164.80	145.50	165.94	157.69	3.70
Day 35							
Villus height, µm	364.13	396.07	324.23**	387.44	310.00	336.54	25.33
Crypt depth, µm	343.56***	205.10	230.11**	164.23	206.80*	166.91	5.83
Day 42							
Villus height, µm	379.44	ND	440.08	ND	358.65	ND	18.32
Crypt depth, µm	322.66	ND	223.39	ND	174.81	ND	4.22

SP- suckled piglets, GP- gnotobiotic piglets, ND- not detectable
*p < 0.05, **p < 0.01, ***p < 0.001

Table 7. Effect of age and weaning on small intestinal morphology at various locations along the intestinal tract of suckling piglets and gnotobiotic piglets

age ($p < 0.01$). Comparison of both animal groups showed that villi in the jejunum of conventional piglets were higher between days 14 and 28 of age, the difference being significant on day 14 ($p < 0.05$). In the post-weaning period, villi were significantly higher ($p < 0.05$) in gnotobiotic piglets on day 35 (Figure 4).

When comparing both mentioned groups, higher villi were observed in ileum of gnotobiotic piglets, resembling the situation in duodenum, with significant difference ($p < 0.05$) on days



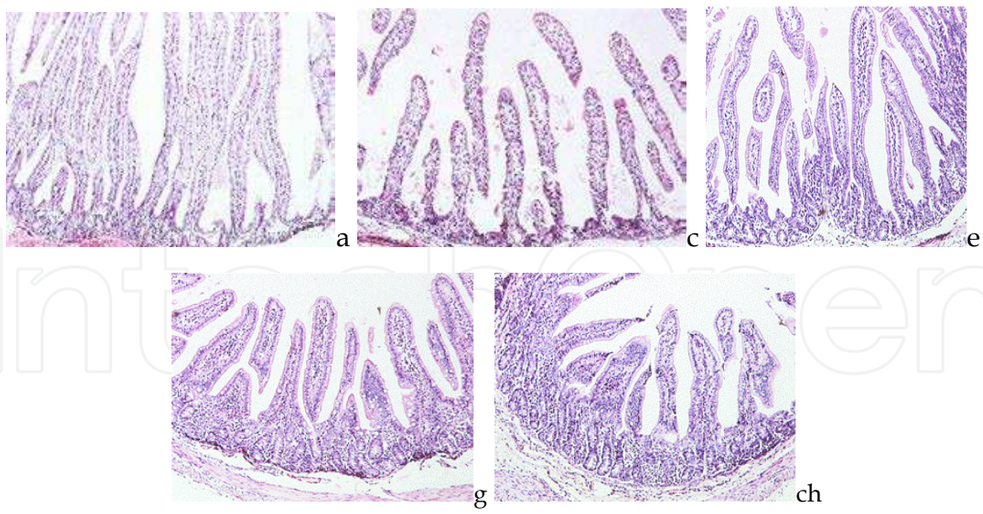
SP- suckled piglets, GP- gnotobiotic piglets
Significantly different (SP vs GP): a ($p < 0.05$), b ($p < 0.01$), c ($p < 0.001$)

Fig. 4. Effect of diets on small intestinal morphology at various locations along the intestinal tract of suckling and gnotobiotic piglets

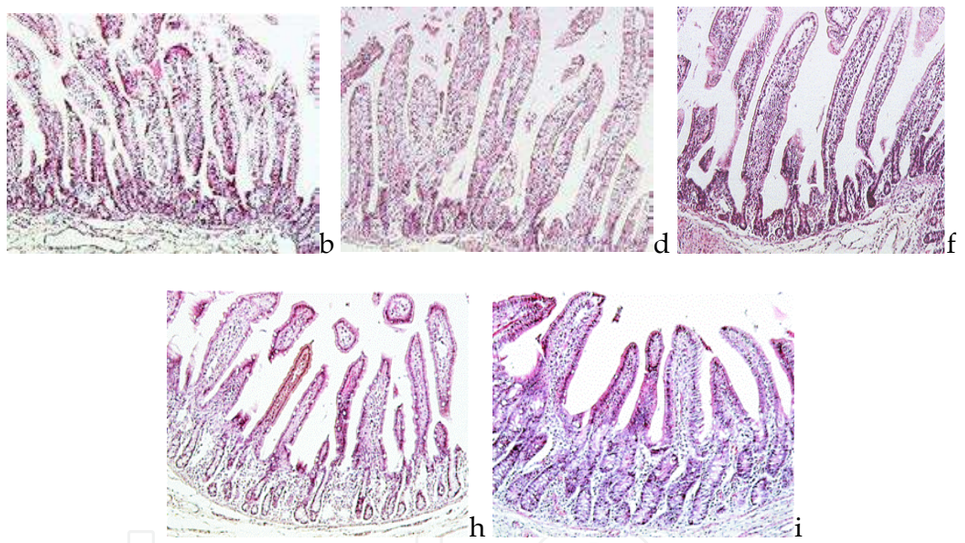
14 and 21 of age with the exception of day 2 of life when ileal villi were significantly higher ($p < 0.001$) in conventional piglets (Figure 4).

The postnatal changes in the depth of crypts (Table 7) were the same in all small intestinal segments of gnotobiotic piglets. From the birth up to day 35 of life the crypts in the jejunal segment gradually deepened and reached 164.23 µm on day 35 of life. Similar development was observed also in the duodenal segment of the small intestine, where, with the exception of slight decrease on day 14 of age, the depth of crypts gradually increased throughout the observation period with significant difference on day 21 of age ($p < 0.05$). One week after weaning the depth of crypts in the duodenum reached 205.1 µm. A significant increase in the depth of crypts ($p < 0.05$) was recorded on day 21 of age also in the ileal segment of gnotobiotic piglets. Also dynamics of development of the depth of crypts was similar in all small intestine segments of colostral piglets. In the first post-weaning week we recorded a significant deepening of crypts in duodenal ($p < 0.001$), jejunal ($p < 0.01$) and ileal ($p < 0.05$) segments.

Staining with haematoxylin/eosin showed that the small intestinal mucosa of gnotobiotic piglets and suckled piglets at 3 h after birth (Figure 5/a,b) and on day 2 of age (Figure 5/c,d) was covered by population of dense, finger-like villi of the same height. While on the surface of villi of gnotobiotic piglets we were able to observe enterocytes with apically located nucleus, in suckled piglets the enterocytes had apically to medially located nucleus. The fibrous base of intestinal villi was poorly differentiated and the intestinal crypts were small. By day 7 of age of gnotobiotic piglets (Figure 5/e) the height of villi decreased but their diameter increased. In the same period the villi in suckled piglets preserved their finger-like shape with medially to basally located nucleus in enterocytes (Figure 5/f). At the



Gnotobiotic piglets



Suckled piglets

(a, b) 3 hours after birth, (c, d) 2 days of age, (e, f) 7 days of age, (g, h) 28 days of age, (ch) 35days of age of GP, (i) 42 days of age of SP, SP- suckled piglets, GP- gnotobiotic piglets, Magnification x 125.

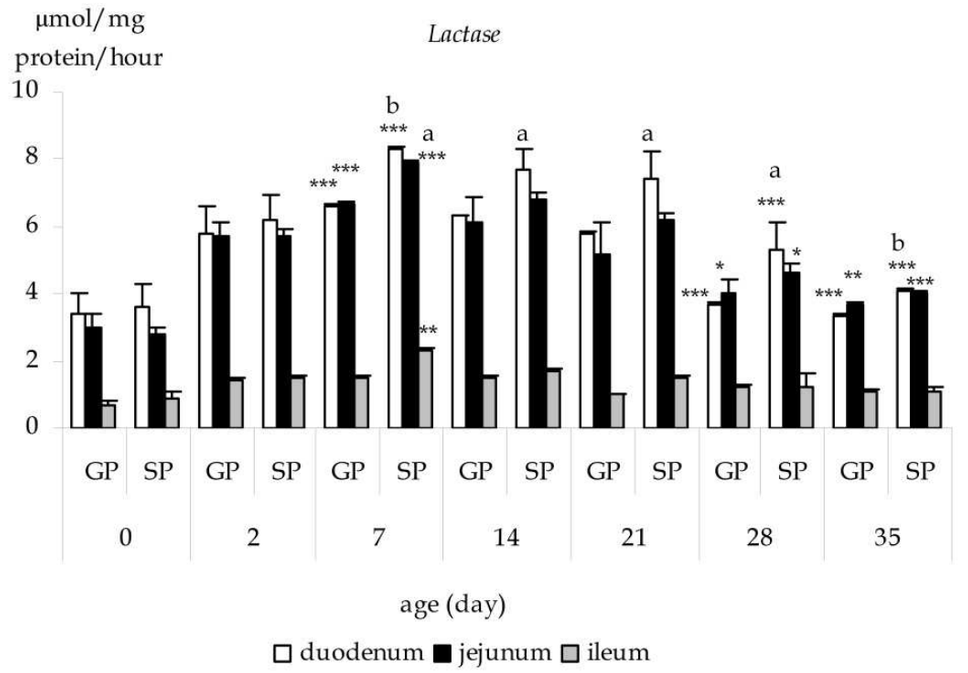
Fig. 5. Light micrograph of hematoxylin and eosin-stained jejunal mucosa of gnotobiotic and suckling piglets

time of weaning (day 28 of age) the differentiated basis of intestinal villi in both observed groups of piglets consisted of thin fibrous tissue containing fascicles of smooth muscle cells. On the surface of the villi we were able to observe goblet cells interspersed among enterocytes (Figure 5/g, h). The enterocyte nuclei were located in the medial part of the cytoplasm. The villi stroma was infiltrated with small number of lymphocytes and plasmatic cells. By day 35 of life, the jejunal villi acquired tongue-like shape (Figure 5/ch). On day 42 of age intestinal crypts of suckled piglets (Figure 5/i) almost completely filled up *lamina propria* of the small intestine and their bases almost reached *lamina muscularis mucosae.*

Intestinal villi were shorter and acquired tongue-like shape. Such characteristic development was observed in all investigated segments of the small intestine which, however, differed by morphometric parameters of villi height.

3.7 Effect of age, weaning and diets on development of specific activity of disaccharidases in the small intestine of gnotobiotic and conventionally bred piglets

In our study we registered that development of lactase activity of gnotobiotic piglets was similar in the duodenum and jejunum in the period from day 0 to 35 of age. The specific activity of lactase (Figure 6) in the duodenum reached at birth the level of 3.4 μmol/mg protein/hour. From the 2nd day of life the increasing enzyme activity was noticed with a measured maximum on day 7 of life ($p < 0.001$). High enzyme levels were observed until the 21th day of life (5.80 μmol/mg protein/hour), after which the enzyme activity decreased in the weeks 4 ($p < 0.001$) and 5 ($p < 0.001$) to levels similar to those noticed at birth. In terms of lactase activity distribution throughout the small intestine, higher activity in the duodenum and jejunum were noticed than in distal parts of the small intestine, where the incidence of enzyme was 4 times lower. The specific activity of lactase in the duodenum of conventional piglets (Figure 6) reached at birth 3.6 μmol/mg protein/hour. The course of activity of this enzyme during our observations resembled that of gnotobiotic piglets with maximum recorded on day 7 of age ($p < 0.001$), gradual decrease from day 14 of life and significant decrease ($p < 0.001$) on the day of weaning and in the 1st post-weaning weak. As far as the distribution of lactase in the small intestine was concerned, the highest activity of lactase

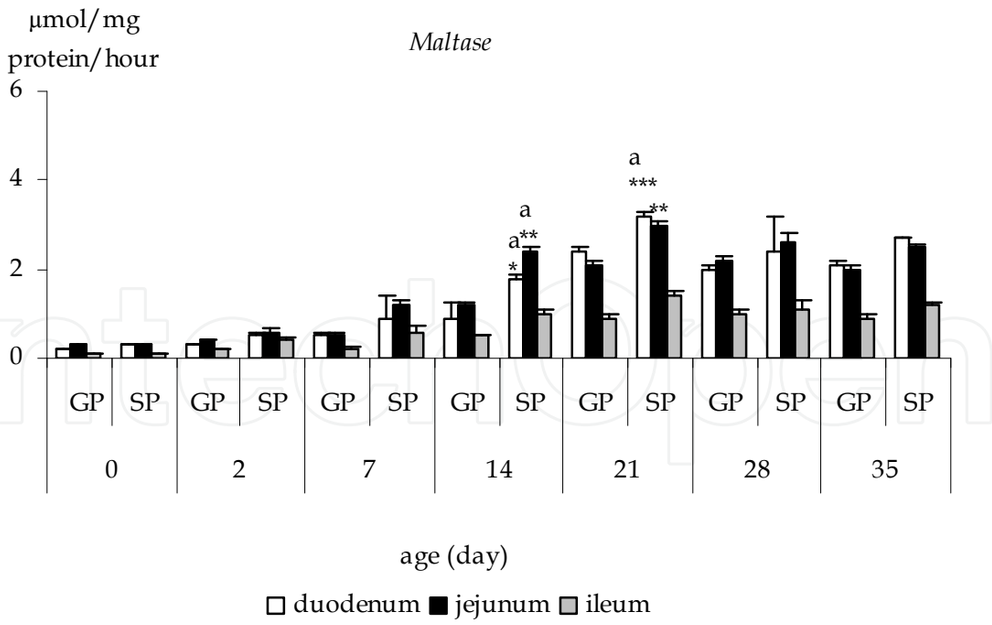


Significantly different (SP vs GP): a ($p < 0.05$), b ($p < 0.01$), *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

Fig. 6. Effect of age, weaning and diets on specific activity of lactase at various locations along the intestinal tract of suckling and gnotobiotic piglets, SP- suckled piglets, GP- gnotobiotic piglets

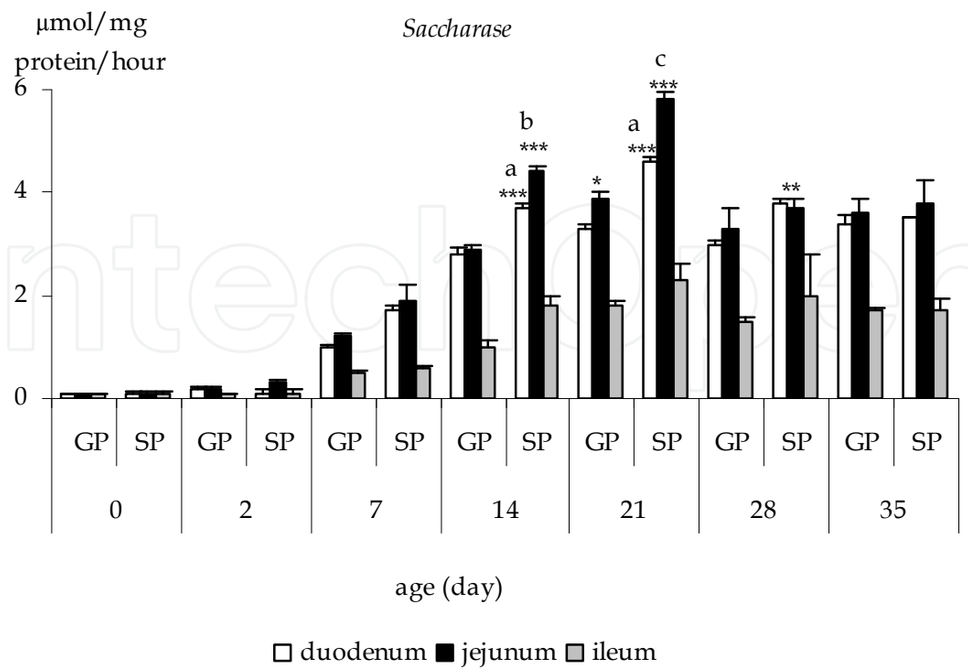
was recorded in duodenum and the lowest in ileum where lactase levels were 4-fold lower compared to proximal small intestine segments. Significant increase in the activity of enzyme was observed in jejunum ($p < 0.001$) and ileum ($p < 0.01$) of conventional piglets on day 7 of age. Comparison of both animal groups (Figure 6) showed that specific activity of lactase in the intestinal tract was higher in conventional piglets throughout the experiment with significantly higher lactase activity in duodenum from day 7 to 35 of age ($p < 0.05$, $p < 0.01$) and significantly higher level in the jejunum ($p < 0.05$) on day 7 of age.

The post-natal dynamics of maltase (Figure 7) and saccharase (Figure 8) in gnotobiotic piglets took a completely different course. Very low levels of maltase in the jejunum detected at birth (0.3 μmol/mg protein/hour) and during the 1st week of life slightly increased in the following period and on the 14th day reached 1.2 μmol/mg protein/hour. The slightly increased specific activity of maltase persisted also in the following period. Maltase distribution (Figure 7) throughout the small intestine changed depending on the age with a predominant concentration of maltase activity in the jejunum at the age of 1-2 weeks, through a higher activity in the duodenal part at the age of 21 days, until a balanced distribution in the proximal and medial part of the small intestine was achieved at the age of 4-5 weeks. Similarly also very low specific saccharase activity in the jejunum (Figure 8) recorded until the 1st week of life (1.12 μmol/mg protein/hour) progressively increased with a maximum on the 21st day of life ($p < 0.001$), sustained values were reached in the 4th and 5th week of the life of piglets. Saccharase activity distribution throughout the small intestine of gnotobiotic piglets was higher in the jejunum during the entire period of observation. Specific activities of maltase (Figure 7) and saccharase (Figure 8) in newborn



Significantly different (SP vs GP): a ($p < 0.05$), *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

Fig. 7. Effect of age, weaning and diets on specific activity of maltase at various locations along the intestinal tract of suckling and gnotobiotic piglets, SP- suckled piglets, GP- gnotobiotic piglets.



SP- suckled piglets, GP- gnotobiotic piglets.
Significantly different (SP vs GP): a ($p < 0.05$), b ($p < 0.01$), c ($p < 0.001$), *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

Fig. 8. Effect of age, weaning and diets on specific activity of saccharase at various locations along the intestinal tract of suckling and gnotobiotic piglets

and 2-day old conventional piglets were very low and did not exceed 0.6 µmol/mg protein/hour. After one week of age of piglets, activities of both enzymes increased. The maximum specific activity of maltase peaked on days 14 and 21 ($p < 0.05$, $p < 0.001$) in the duodenum and significant difference was observed ($p < 0.05$) in the jejunal segment. Similarly, activity of saccharase reached highest values on days 14 and 21 of age and differed significantly ($p < 0.001$) in both the duodenal and jejunal segment. On day 28 of age we observed a decrease in the specific activity of both enzymes with significant difference in saccharase ($p < 0.01$) in the jejunum. Observation of distribution of maltase in the small intestine of conventional piglets showed that in the direction of terminal ileum, up to the age of 2 weeks the highest activity was detected in the jejunum and the lowest in the ileum. In the following period activity of this enzyme was higher in proximal to medial segments of the intestine than in the distal ones. Throughout the experiment saccharase reached the highest activity in the jejunum and the lowest in the ileum.

The postnatal development of specific activity of enzymes maltase and saccharase showed a similar trend with higher activities of both enzymes in suckled piglets in all digestive tract segments throughout the observation. The highest activity of the enzymes in comparison with gnotobiotic piglets was recorded on days 14 and 21 of age with significant differences in the level of maltase ($p < 0.05$, $p < 0.001$) in the duodenum and significantly different activity of the enzyme ($p < 0.05$) in jejunum. Significantly different activities of saccharase compared to the other group of piglets were observed in the same period (days 14 and 21) in duodenum ($p < 0.05$) and jejunum ($p < 0.01$, $p < 0.001$).

4. Discussion

4.1 Effect of age and diets on development of the small intestine – Intestinal microflora

At birth, a young pig which is axenic during its uterine life is suddenly confronted with a complex bacterial environment. Beginning with birth, the young are in contact with several microbial ecosystems - i.e. faeces, contaminated vagina and perineum, but also from the skin and teats of the sow which are usually contaminated as well. It could be assumed that each of these ecosystems contributes to constituting the gut flora of the newborn (Siggers et al., 2007). However, in the following days, simplified microbiota profiles have been characterized, which will become more complex with time, increasing its diversity as the animal grows (Inoue at al., 2005). The population level of the microbiota in various parts of the gastrointestinal tract of monogastric animals depends on the attachment ability, replication period of the microorganism under the physicochemical tract conditions and the emptying rhythm in the part of the gastrointestinal tract under investigation. Swords et al. (1993) studied pig faecal microbiota evolution within the first four months of life, and concluded that the establishment of the adult faecal flora is a large and complex process with three different marked phases in the bacterial succession. The first phase corresponds with the first week of life, the second one, from the end of the first week to conclusion of suckling, and the third phase from weaning to final adaptation to dry food. In this first phase, aerobes and facultative anaerobes from the sow and the environment become the predominant bacterial groups, comprising 80% of the total flora by three hours after birth. The gut colonization is extremely fast, only twelve hours after birth, total bacteria in distal colon reaches counts of 10^9 cfu/g colonic content (Jensen et al., 1998; Swords et al., 1993). First colonizers modify the gastrointestinal environment (by consumption of molecular oxygen and reduction of the redox potential), making it more favourable for the following colonization by anaerobes. As a result, aerotolerant bacteria are gradually supplanted by strict anaerobes, and 48h after birth, piglets already show 90% of anaerobic bacteria (Swords et al., 1993). Of these bacterial groups, lactobacilli and streptococci become the dominant bacteria at the end of the first week of life and will be maintained for the whole suckling period with counts of around 10^7-10^9 cfu/g digesta (Swords et al., 1993). Microbiota remains fairly stable in terms of species composition during the second phase when the piglets receive milk from their mother (Mathew et al., 1996). The diversity of anaerobic bacteria increases in this period (Inoue et al., 2005) and supplantation of aerobic and facultative anaerobic bacteria by anaerobic bacteria become almost completed in this phase. As has been mentioned before, lactobacilli and streptococci continue being dominant bacteria, which are well adapted to utilize substrate from the milk diet. *Clostridium*, *Bacteroides*, *bifidobacteria*, and low densities *Eubacterium*, *Fusobacterium*, *Propionibacterium* and *Streptococcus* spp. are also usually found in this second phase (Swords et al., 1993). In our experiment with piglets fed maternal milk, the gut flora developed very quickly post partum. As early as within 48 hrs post partum, *Escherichia coli*, enterococci and lactobacilli were detected in the content of digesta in suckled piglets, the populations of which represented 10^5 - 10^9 bacteria/g per sample. The gut flora of piglets in the proximal part (jejunum) consisted of facultatively anaerobic bacteria (enterococci, *E. coli*) and aerotolerant anaerobes (lactobacilli) counting 10^4 - 10^6 bacteria/g per sample. These numbers increased progressively in the ileum and the dominant flora in the posterior portions of the digestive tract (caecum) was facultatively anaerobic bacteria (*E. coli*, enterococci, *Enterobacteriaceae* and lactobacilli) counting 10^8 - 10^9 bacteria/g per sample. As

the piglets grew, the flora progressively changed. The replacement of maternal milk by a milk replacement diet in our third experiment resulted at 2 and 14 days of age in increased numbers of bacterial flora, of the family *Enterobacteriaceae* in the proximal small intestine (jejunum) by 3 log compared to suckled piglets. At the same time a gradual increase in the numbers of coliform bacteria ($p < 0.001$) in the proximal (jejunum) and terminal (caecum) part of the intestine occurred reaching numbers as much as by 4 log higher in the jejunum at 14 d of age compared to colostral piglets. Similar findings were obtained by (Franklin et al. 2002; Jensen et al., 1998; Pluske et al., 1997) which observed, in addition to higher populations of anaerobic and coliform bacteria, a significant decline in lactobacilli populations in piglets fed replacement milk. The mechanism of the selection has not yet been determined, although feeding may be a primary factor. According to different authors it is almost impossible to prevent neonatal *E. coli* diarrhoea in piglets which do not receive maternal colostrum (Pluske et al., 1997; Xu et al., 1996). Colostrum and also maternal milk contain highly digestible nutrients and components such as immunoglobulins and lysozymes and have both bacteriostatic and antiadhesive properties towards the pathogen *E. coli* (Xu et al., 1996). This protective effect, however, does not seem to be accompanied by an elimination of *E. coli* from the digestive tract. In addition, in both the jejunal and ileal part ($p < 0.001$) of the small intestine of replacer-fed piglets, high numbers of enterococci were recorded, being by 1-3.5 log higher compared to the colostrum-fed piglets throughout the period of observation. It is likely, however, that this result was influenced by the milk replacement having been enriched by *Enterococcus faecium* counting 10^4 cfu/g of feed. The human intestinal microbiota is a complex ecosystem, consisting of several hundred (more than 800) different bacterial species. This microbiota plays an important role in human health and nutrition by producing nutrients, preventing colonization of the gut by potential pathogenic microorganisms (Guarner & Malagelada, 2003), and preserving the health of the host through interactions with the developing immune system. The microbiota in early life has been linked to allergy risk (Penders et al., 2007). Major changes in the intestinal microbial composition occur in early life. Sterile *in utero*, the gastrointestinal tract of the newborn infant is rapidly colonized at birth by a myriad of maternal vaginal and faecal bacteria and other sources from its environment. The first few weeks after birth correspond to critical stages of gut colonization. Bacterial colonization of the gastrointestinal tract is influenced by numerous factors including diet, environment, antibiotic treatment, mucosal maturation, and age. Naturally delivered babies experienced a period of 2-3 days in which, as a consequence of the low selective potential of their stomach and small bowel, bacteria invading and reproducing within the gut belong to aerobic species as *Enterobacteriaceae*, streptococci, and staphylococci. These bacteria, arriving from the external environment, belong to species with a pathogenic potential, and therefore, it might seem that they would not be the best choice for the health of neonates. However, the metabolisms of these bacteria are believed to be positive factors in preparing the path to a beneficial enteric flora. In the study of Fallani et al. (2010) they confirmed previously published work (Hopkins et al., 2005; Penders et al., 2007) that bifidobacteria are the predominant group detected in the faeces of pre-weaned infants, followed by *Bacteroides* and enterobacteria.

4.2 Production of SCFAs and pH

Organic acids are the main metabolites of intestinal fermentation. The degree of their concentration in the digesta reflects the level of intestinal fermentation (Piva et al., 2002). It is

well known that organic acids exhibit antibacterial activity (Piva et al., 2002), increase intestinal absorption of minerals and improve ileal digestion of proteins and amino acids. Their relative levels vary depending on location (stomach, small intestine, large intestine) and diet composition (dietary lactose level, fibre level, etc). Lactic acid is in the greatest concentration in the stomach and small intestine while other organic acids, acetic along with propionic and butyric, are predominant in the large intestine. In our study, in suckled piglets we recorded higher level of acetic acid in the ileal contents in comparison with the replacer-fed piglets throughout the observation, ranging from about 7 to 21.5 mmol/l. Higher concentrations of this acid were observed in the same segment also in gnotobiotic piglets compared to replacer-fed piglets, ranging from 2.05 to 13.69 mmol/l. A similar tendency was recorded in lactic acid, in the ileal content, with higher concentrations in suckled piglets compared to the replacer-fed piglets in which the values later ranged from about 8.2 to 21.4 mmol/l. Higher production of lactic acid in gnotobiotic piglets in comparison with replacer-fed piglets was recorded throughout the observation and ranged from 0.6 to 10.14 mmol/l. Increasing concentrations of lactate in ileal digesta should therefore reflect an increased population and activity of lactic acid bacteria (Pluske et al., 2002). These findings are of importance relative to the management of growing pigs, because lactate has been shown to have antibacterial effects on *E. coli* and *Salmonella* species, and lactobacilli have been shown to inhibit adhesion of enterotoxigenic *E. coli* to the ileal epithelium (Pluske et al., 2002). The ability to generate organic acids, particularly lactic and acetic acid, present one of the mechanisms by which lactobacilli perform their inhibitory effect upon pathogens. With decreasing pH values, the inhibitory activity of the above acids increases, their molecular form being toxic for bacteria. The increased toxicity of acetic acid is attributed to its higher pKa in comparison to lactic acid. Increased lactic acid levels intensify the toxicity of acetic acid. Comparison of lactic acid levels in the jejunal and ileal contents of one week old gnotobiotic piglets (Bomba et al., 1998) and conventional suckling piglets (Zitnan et al., 2001) revealed that the highest levels were found in conventional animals (29.30 and 27.90 mmol/l, resp.) and in *Lactobacillus plantarum* inoculated gnotobiotic piglets (26.60 and 14.20 mmol/l, resp.). High levels of lactic acid were recorded in our study in jejunal and ileal contents of conventional suckling piglets (27.52 and 26.91 mmol/l, resp.) and piglets inoculated with *Enterococus faecium* at the age of 1 week (23.59 and 24.56 mmol/l, resp.). Lower concentrations of this acid were found in replacer-fed piglets (24.92 and 14.42) and the lowest levels of lactic acid in the jejunal and ileal contents (Bomba et al., 1998) were seen in germ-free piglets (4.40 and 6.45 mmol/l, resp.). At the age of 3 weeks, the level of lactic acid in the jejunum of piglets inoculated with *Enterococcus faecium* was lower in comparison with that in the jejunum of piglets inoculated with *Lactobacillus plantarum* (21.94 and 33.15 mmol/l, resp.) but in the ileum of *Enterococcus faecium* inoculated piglets we found the highest level of this acid (24.40 mmol/l) in comparison with all other groups of piglets. The mentioned authors presented different results also with regard to acetic acid, as the highest concentrations of acetic acid in the jejunum and ileum (30.05 and 23.61 mmol/l, resp.) were observed in conventional piglets by Zitnan et al. (2001) and in conventional suckling piglets investigated in our study (33.05 and 21.81 mmol/l, resp.). Lower levels were observed in replacer-fed piglets (21.43 and 10.47 mmol/l, resp.) and in gnotobiotic piglets (Bomba et al. 1998) inoculated with lactobacilli (11.80 and 11.85 mmol/l, resp.) and in those inoculated with *Enterococcus faecium* (8.18 and 24.16 mmol/l, resp.). Similarly low levels were detected also in germ-free piglets (13.15 and 3.9 mmol/l, resp.). On the contrary, at the age of 3 weeks, we recorded higher levels of acetic acid in the jejunal and ileal content of

gnotobiotic piglets inoculated with *Enterococcus faecium* (12.86 and 32.08 mmol/l, resp.) in comparison with all other groups of piglets (10.7 and 25.9 mmol/l, resp.) of conventional suckling piglets (Zitnan, 2001), (5.36 and 25.00 mmol/l, resp.) of conventional suckling piglets in our study, (6.87 and 19.86 mmol/l, resp.) of replacer-fed piglets and gnotobiotic piglets inoculated with *Lactobacillus plantarum* (11.85 and 14.2 mmol/l, resp.). Under the influence of more diverse populations of microorganisms the conditions in the colonic content changed with gradual occurrences of propionic, butyric, and valeric acids. Organic acids and ammonia concentrating in the colon were according to Kiare et al. (2007) 5-10-fold bigger than those in the ileum and their increased concentrations resulted in additional fermentation activity of short-chain organic acids. This indicates higher microbial activity and considerable N-metabolism in the caudal segment of the intestine. The most significant increase in production of organic acids in the colonic segment observed in our study involved acetoacetic, acetic, propionic and butyric acids in the group of suckled piglets compared to replacer-fed piglets, at important concentrations of acetoacetic acid from 14 to 28 days of age ($p < 0.01$). Significant increase was observed also in production of lactic acid in gnotobiotic piglets compared to the replacer-fed animals throughout the observation period with the highest concentrations reached on days 2 ($p < 0.05$), 14, 21 and 28 of age ($p < 0.01$). Decreased pH of the gut content and increased production of lactic and acetic acids affects positively optimisation of digestive processes. Bomba et al. (1998) investigated intestinal metabolism of gnotobiotic piglets and recorded significantly lower pH ($p<0.05$) in the jejunal content in piglets inoculated with *Lactobacillus plantarum* in the 1st week of life in comparison with germ-free piglets. Zitnan et al. (2001) observed pH in the jejunal and ileal content of conventional piglets of the same age. When comparing the actual acidity in individual segments of the small intestine, pH of the jejunum content of germ-free piglets was higher in the first week of life (7.49) in comparison with pH of conventional piglets (6.23) of the same age. Contrary to that, pH of the jejunal content of gnotobiotic piglets inoculated with *Lactobacillus plantarum* was considerably lower (5.63). Similar low pH was recorded in our study in gnotobiotic piglets of the same age inoculated with *Enterococcus faecium* (6.02). Bomba et al. (1998) conducted two experiments to investigate the influence of short-term and continuous preventive administration of *Lactobacillus casei* subs.casei against *E.coli* on actual acidity, production of organic acids and colonisation of jejunum with *E.coli* O8:K88 in gnotobiotic piglets. After the short-term administration of *L.casei* they recorded lower pH in the jejunal content of experimental piglets (L-E) while pH of the ileal content of these piglets increased significantly (7.63) in comparison with the control (7.03). After continuous administration, the authors recorded lower pH in the experimental group L-E (6.1) in comparison with the control group (6.28). In our experiment we observed a positive influence of *Enterococcus faecium* on intestinal metabolism in replacer-fed piglets in terms of increased production of organic acids (formic acid, acetoacetic, propionic and butyric acid). However, production of lactic acid responsible for decrease in pH was lower in our observations and their concentrations ranged between 6.27 and 20.30 mmol/l in the ileal segment of replacer-fed piglets in comparison with suckled piglets (15.52 to 60.11mmol/l) and failed to induce the corresponding pH reduction. pH in the ileum of non-colostral piglets ranged from 6.9 to 7.6. Although acetic acid reached higher levels in the colon segment, it could become toxic only at low pH of the environment dependent on sufficient concentration of lactic acid in the gut. According to Mufandaedza et al. (2006), decreased active acidity, $pH < 5.0$, limits even stops growth and multiplication of *E. coli*. Similar conclusions were drawn from our study. Production of organic acids by replacer-fed piglets

was low and resulted in their low concentrations which did not decrease pH so effectively as it was observed in gnotobiotic piglets inoculated with *Enterococcus faecium.* Deficit of colostral nutrition in these piglets resulted in worsened health in 8 out of total 26 piglets. The disease was peracute and proceeded with physiological temperature. Even though antibiotics were administered, the piglets died within 8 hrs of appearing of the first symptoms. In the piglets, lymphocytic leukocytosis as well as hypochromic anemia were diagnosed, and *E. coli* K88 was isolated from rectal swabs.

4.3 Intestinal morphology and disaccharidase activity

Due to numerous similarities of the physiology and anatomy of the gastrointestinal tract of man and pigs, the pig model is a very attractive model for human nutritional studies (Miller & Ullrey, 1987). Investigations performed in humans and pigs showed that the portions of total life required to reach chemical maturity for both these species are nearly identical, 4.4% and 4.6, respectively. Even though there are species-specific differences of the placenta and immunological system of pigs and human, the piglets are optimum experimental model for investigations concerning physiology and pathology of the gastrointestinal tract of human newborn (Miller & Ullrey, 1987). The postnatal development of the gastrointestinal (GI) system is a very dynamic process. In the neonatal pig with the mean birth body weight of 1.45kg, the small intestine and pancreas weight contribute to 3.1% and 0.14% of the total body weight, respectively (Zabielski et al., 2008). Within the first four postnatal weeks weight of the piglet is increased >5-fold, with the GI organs growing faster than many other organs of the body (Zabielski et al., 2008). Can we suspect the same changes in human neonates? Presumably yes, but the intensity of the remodelling is not as dramatic. The development in humans is slower, and the growth rate is slower in comparison to pigs. In humans the birth weight is doubled within ca. 170 days. Nevertheless, a number of similarities pig and human in the process of the development can be seen. In the study of Len et al. (2009), similar to our investigations of conventional piglets, the absolute weight of visceral organs and GI tract increased with piglet age. However, when expressed as g/kg empty body weight, the weight of visceral organs decreased with age (Len et al., 2009), which is in agreement with Pluske et al. (2002), who found that the relative weight of the visceral organs of piglets had a tendency to decrease between 14 and 28 days of age. In our study we observed a gradual decrease in the weight of small and large intestine between days 2 and 21 of age in suckled piglets while in gnotobiotic piglets the relative weight of intestines increased gradually throughout the period of observation. In germ-free and monoassociated pigs (Shirkey et al., 2006), the relative small intestine length was reduced compared with conventional pigs. The mechanisms affecting intestinal length are unknown, however, it can be hypothesized that increased small intestine length in conventionalized pigs is a compensatory response to the decreased absorptive capacity associated with decreased surface area (decreased villi length) and/or to direct competition with the microbiota for dietary nutrients. Shirkey et al. (2006) observed that in the proximal region of the small intestine, the relative weights for segments from conventional pigs tended to be higher than those from germ-free and monoassociated pigs. This is consistent with our study, as well as with the previous reports indicating that compared with germ-free animals, conventionally reared animals experience intestinal "thickening" associated primarily with increased *lamina propria* cellularity (Miniats & Valli, 1973) as well as thickening of the submucosa and muscular layers (Furuse & Okumura, 1994). On the

contrary, higher relative weight of the distal part of the intestine was reported in germ-free piglets (Shirkey et al., 2006). Similar results were obtained in our study which showed higher relative weight of the large intestine in gnotobiotic piglets inoculated with *Enterococcus faecium* in comparison with conventional piglets starting from the second week of age up to the weaning (day 28 of age). In addition to an intensive growth of the GI system, during the first month of life an intense rebuilding of the tissues takes place. The most intensive processes are observed in the epithelium of the small intestine (Zabielski et al., 2008). The weight of small intestinal mucosa doubles during the first postnatal day due to a complex of processes involving, accumulation of colostrum proteins in the enterocytes as a result of an open „gut barrier", increase of local blood flow concurrently with a reduction in basal vascular resistance (Nankervis et al., 2001), and finally changes in epithelial cell turnover, namely, increased mitosis accompanied by the inhibition of apoptosis which result in a 2-fold increase in the mitosis/apoptosis ratio within the first 2 postnatal days (Zabielski et al., 2008). The regulation of small intestine development (especially the tissue growth) is in a positive feed-back to colostrum and milk intake (Marion et al., 2003). Currently none artificial feeding system (milk, artificial milk formula, nor feeding with any other compositions like lactose, glucose solutions) could reproduce the developmental characteristics obtained with maternal colostrum feeding (Zabielski et al., 2008). Furthermore, high specificity of colostrum, especially concerning the composition of hormones and bioactive compounds prevents utilization of colostrum of other species as the replacement. In the study of Meslin et al. (1973), the overall mass of the small intestine in germ-free species was decreased, and its surface area was smaller, whereas the villi of the small intestine were unusually uniform in shape and appear slender, with crypts, which were shorter and less populated than in the respective conventional control animals. Our study showed that the jejunal part of the intestinal tract in gnotobiotic pigs was characterized up to 14 days of life by relatively short crypts, extremely long villi and narrow *lamina propria* containing few cells. Reduced crypt depth and increased villus length agree with the previous observations in germ-free pigs (Shirkey et al., 2006; Shurson et al., 1990). In the present study in gnotobiotic piglets villi were the longest in the jejunum and shortest in the duodenum and ileum, whereas crypt depth was shortest in the jejunum and deepest in the duodenum throughout the observation period. These morphological characteristics suggested that the rates of enterocyte proliferation and exfoliation were the highest in the proximal small intestine, as indicated by deep crypts and shorter villi, respectively, with rates decreasing distally along the small intestine (Hampson & Kidder, 1986). In agreement with our morphological findings, Miniats & Valli (1973) reported longer jejunal villi in germ-free pigs but did not measure villi in other regions. Shurson et al. (1990) reported that germ-free pigs had longer ileal and duodenal villi but shorter jejunal villi compared to their conventional counterparts. Similar results were obtained also in our study as the villi in the duodenum and ileum of gnotobiotic piglets were higher in comparison with conventional piglets throughout the experiment. The difference was significant at 3 hours after birth, on days 2 and 7 of age ($p < 0.01$) and day 14 of age ($p < 0.05$) in the duodenum and on days 14 and 21 in the ileum ($p < 0.05$). Shirkey et al. (2006) suggested that regional variation in morphology, especially in the proximal small intestine, is not entirely dependent on microbial colonization but is also influenced by such non-microbial factors as bile salts, pancreatic secretions, and compounds of dietary origin which would be expected to be in higher concentration and have more contact with mucosal surface in the duodenum.

The gastrointestinal tract goes through substantial structural and functional changes in the early postnatal period (Walthall et al., 2005). As the piglets grow, functional changes occur in the expression and kinetics (Fan et al., 2002) of brush border digestive enzymes. Each brush border enzyme shows a specific developmental pattern as the animal ages, which have been associated with the maturation of enterocytes (Walthall et al., 2005). Specifically, changing disaccharidasae activities have been used as an indicator of intestinal maturation. Measurable lactase levels were detected in bush border homogenates and membrane vesicles of the small intestine in the 7th week of pregnancy (Buddington & Malo, 1996). For comparison, activity of lactase in human foetuses was confirmed only later and only in the 34th week of gravidity (Menard & Basque, 2001). Aumaitre & Corring (1978) measured lactase in small intestine homogenates from pig foetuses at 105th day of gravidity and observed that the total activity of lactase in the intestine amounted to only 10% of the activity determined at birth. It was stated that specific activities of lactase in homogenates or membrane vesicles of the small intestine brush border were high at birth and stayed at this level during the first 7-10 days of postnatal life (Torp et al., 1993). In suckling pigs, lactase activity was observed to undergo an initial marked decrease sometime during the second to fifth week of age which was followed by a period when it remained relatively constant or continued to decrease gradually up to 8 weeks of age (Kelly et al., 1991). In terms of enzyme distribution throughout the intestine, neonatal and 1-day old piglets show the highest specific lactase activity in the proximal part of the small intestine and the lowest in the distal part, but at the age of 6-10 days its distribution throughout the intestine was more regular (Buddington & Malo, 1996). The intestinal microbiota has been shown to affect brush border enzyme expression, as the intestine of a germ-free mouse has a different pattern of brush border enzymes than a conventional mouse (Kozakova et al., 2001). The mechanism by which bacteria induce changes in brush border enzyme activities or which bacteria are responsible has not been elucidated. According to (Willing & Kessel, 2009), conventionalization in pigs reduced enterocyte brush border enzyme activity compared with germ-free without a concomitant reduction in gene expression in the case of lactase phlorizin hydrolase. Because of the reduced villus height and increased enterocyte replacement rate observed in conventional as compared with germ-free animals (Furuse & Okumura, 1994), it has been postulated that the higher disaccharidase activity in the small intestine of germ-free as compared with conventional rats is because of an increased number of mature enterocytes (Willing & Kessel, 2009). These reports were confirmed by Reddy & Wostmann (1966) who observed that disaccharidase activity was higer in the small intestine of the germ-free as compared with conventional rats. However, in our study, contrary to previous studies, specific activities of lactase along the entire intestinal tract were higher in conventional piglets throughout the experiment. Kozakova et al. (2001) concluded that individual bacteria can stimulate a similar response, as monoassociation of gnotobiotic mice with *Bifidobacteria bifidum* induces a shift in enzyme activity to a pattern similar to that of a conventional mouse. Aumaitre & Corring (1978) reported that the intestinal tract of foetal (105th day of gravidity) and newborn piglets contained maltase but saccharase was present in one week old piglets. Similar observations for saccharase were presented by Buddington & Malo (1996). However, the studies by James et al. (1987) and Sangild et al. (1991) revealed low activities of saccharase and maltase in the small intestine of newborn piglets. Starting from 1 week of age specific activities of maltase and saccharase abruptly increased reaching maximum at the age of 10 - 16 days and sustained values at the age of approximately 3 weeks (James et al., 1987; Sangild et al., 1991). Similar tendencies of specific activity of

maltase and saccharase were observed also in our study. When comparing the postnatal development of specific activities of enzymes maltase and saccharase in gnotobiotic and conventional piglets we observed a similar trend but higher activities of both enzymes in all segments of digestive tract of conventional piglets throughout the observation period. In 6 - 7 days old piglets, the distribution of activities of saccharase and maltase was similar along the small intestine but the activities of both enzymes were higher in the proximal to medial parts of the jejunum compared to distal part of the small intestine (Aumaitre & Corring, 1978; Buddington & Malo, 1996). In our study, saccharase activity distribution throughout the small intestine of gnotobiotic and conventional piglets was higher in the jejunum during the entire period of observation. Distribution of maltase along the small intestine changed depending on age, from predominant concentration of the activity in the proximal half of the small intestine at the age of 1-2 weeks (Aumaitre & Corring, 1978) through uniform distribution along the small intestine at the age of 2-3 weeks (Kelly et al., 1991) up to higher activity in the range of 10-15% along 80-90% of the intestine length at the age of 5 - 8 weeks (Hampson & Kidder, 1986). Similar distribution of maltase along the small intestine was observed also in our study in both gnotobiotic and conventional piglets. Transition from milk nutrition to definitive nutrition in children is accompanied with induction of maltase and decreasing activity of lactase as an adaptation of GIT to changes in nutrition with age (Menard & Basque, 2001). Other factors besides age which affect development of disaccharidases activities in the small intestine include: feed offered to piglets in the period of suckling (Hampson & Kidder, 1986), weaning to dry or liquid feed, growth factors, for example epidermal growth factor (James et al., 1987) and hormones, for example insulin (Shulman, 1990), corticosteroids (Kreikemeier et al., 1990), ACTH - adrenocorticotropin (Sangild et al.,1991). Willing & Kessel (2009) concluded that enterocyte upregulation of brush border enzyme expression occurs as either a direct response to microbial colonization or as a feedback mechanisms in response to reduced enzyme activity through microbial degradation. This mechanism may play a role in ensuring effective competition of the host with the intestinal microbiota for available nutrients.

4.4 Effect of weaning on development of the small intestine of conventionally and gnotobiotic bred piglets

Another critical phase in the gastrointestinal tract development of young animals is the weaning period. The weaning of piglets usually takes place between 3 and 4 week of life, when the majority of nutrients are ingested with milk. Weaning for farm animals occurs in an early age, when the gastrointestinal system motility, digestive and absorptive functions are not yet matured and prepared for food other than milk. In a wild boar, domestic pig ancestor, the offspring is weaned in much older age and change of the diet is gradual, therefore weaning disorders are nearly nonexistent. In intensive livestock production shorter suckling period benefits in increased number of piglets born per year, but at the negative side is an increased number of weaning disorders (Zabielski et al., 2008). Weaning is associated with mixing of piglets from different litters and sometimes also with the transport of animals from the place of birth to specialized nursery units. This results in profound social and environmental stress which is a caused also by the changes in the diet. The gastrointestinal tract has to adapt to the new type of feed, which leads to changes in myenteron motility, enzymes secretion and activity, and the composition of bacterial flora (Barszcz & Skomial, 2011). According to Lalles et al. (2007) weaning is a critical phase for

piglets, it is associated with a variable period of anorexia during the first days after weaning, the deterioration of the digestive function and accumulation of undigested feed as a result of inefficient digestion. During this period, piglets are more susceptible to suffer from post-weaning diarrhoea with the proliferation and attachment to the intestinal mucosa of β-haemolytic strains of *E. coli* (Fairbrother et al., 2005). Nabuurs (1998) and Pluske et al. (1997) stated that predisposition to infections with eneterotoxigenic bacteria depends on a number of factors. Miller et al. (1986) concluded that the problems induced by weaning were caused rather by the changes in the structure of the intestines and specific loss of digestive enzymes than by any great changes in absorption function despite the fact that the data of Nabuurs et al. (1998) were contradictory. Nabuurs (1998) concluded that piglets suffering from post-weaning diarrhoea excreted enterotoxigenic *E. coli* strains and *rotavirus,* and that these piglets developed a hyperregenerative villus atrophy, and subsequently a severe loss of net absorption of fluid and electrolytes in the small intestine. A simulated halving of the absorption in the large intestine of weaned piglets aggravates the adverse effects of an enterotoxigenic *E. coli* in the small intestine (Nabuurs, 1998). Franklin et al. (2002) recorded no post-weaning increase in *E.coli* in pigs weaned at 17 days of age, in agreement with the studies of Etheridge et al. (1984) and Mathew et al. (1998), but in contrast with others (Mathew et al., 1996) who reported increase in *E.coli* populations after weaning. We have observed changes during the first week post-weaning in the jejunal part of the digestive tract of colostral piglets that pointed to a decrease in all observed groups of bacteria with the highest decrease by 1-1.8 log for enterococci, *E. coli* and *Enterobacteriaceae.* Mathew et al. (1998) postulated the absence of an *E.coli* increase may be due to weaning pigs into a highly sanitized, environmentally controlled room with limited contact among pigs. Franklin et al. (2002) also observed *E.coli* populations to be lower in pigs remaining on the sow, as have other investigators (Etheridge et al., 1984; Mathew et al., 1996). Jensen (1998) reported that lactobacilli are inversely proportionate to coliform bacteria during 1 week post-weaning. This is also confirmed by the results of Risley et al. (1992), but however, has not been confirmed in our experiments. In the study by Franklin et al. (2002) faecal populations of lactobacilli and *E.coli* followed patterns typical of those observed in the more anterior portions of the gastrointestinal tract. However, faecal bifidobacteria populations increased post-weaning, possibly due to the decrease in lactobacilli and *E.coli* in the posterior gastrointestinal tract. The loss of direct competition may benefit other bacterial populations, including bifidobacteria. The infant´s microbiota initially shows low diversity and instability, but evolves into a more stable adult-type microbiota over the first 24 months of life (Zoetendal et al., 1998). *Bifidobacterium* populations are dominant in the first months of life, especially in breast-fed infants due to the bifinogenic effect of breast milk, while a more diverse microbiota is found in formula-fed infants, weaning children and adults (Gueimonde et al., 2006). In adults and weaned children the major constituents of the colonic microbiota are *Bacteroides*, followed by several genera belonging to the division *Firmicutes*, such as *Eubacterium*, *Ruminococcus* and *Clostridium*, and the genus *Bifidobacterium*. By contrast, in infants the genus *Bifidobacterium* is predominant and also a few genera from the family *Enterobacteriaceae*, as a *Escherichia*, *Raoultella*, and *Klebsiella* (Kurokawa et al., 2007). It is well know that weaning has a dramatic negative impact on the intestinal mucosal morphology of piglets. Significant post-weaning reduction in villus height has been observed by study (Berkeveld et al., 2007). In study of Hedemann et al. (2003) villus height decreased to a minimum during the first 3 days post-weaning and this is in accordance with the other studies showing that villous height is minimal 2-5 days post-weaning (Hampson & Kidder, 1986; Kelly et al., 1991). In the jejunum and ileum of conventional piglets, investigated in our

study, we observed a post-weaning decrease in the height of villi significant on day 35 of age in the jejunum ($p < 0.01$). Elongation of the crypts post-weaning has been observed in several studies (Hampson & Kidder, 1986; Hedemann et al., 2003) and was confirmed in the present experiment. In our study, in the first week after weaning, conventional piglets showed significant deepening of crypts in duodenal ($p < 0.001$), jejunal ($p < 0.01$) and ileal ($p < 0.05$) segments. Villous atrophy may result both from increased rate of cell loss leading to higher rate of mitosis in crypts and their hyperplasia and from slower rate of cell renewal resulting from the reduction of cell division, i.e. in case of underfeeding. During the time of weaning villous shape also undergoes modifications. The marked and abrupt morphological response to weaning in the small intestine, characterized by transformation from a dense finger-like villi population to a smooth, compact, tongue-shaped luminal villi was observed in previous study (Skrzypek et al., 2005) and in the present study. The morphological changes observed in the small intestine around weaning are closely related to changes in the mucosal enzyme activity observed at the same time. When shortening of the villi is associated with cell loss, loss of mature enterocytes where digestive enzymes are located also occurs. The disaccharidases have been the most commonly investigated mucosal enzymes in relation to weaning of piglets (Kelly et al., 1991). Morphological changes in the small intestine of piglets after weaning are accompanied by smaller activity of brush border enzymes, lactase and sucrase (Pacha, 2000). In our study we registered that of lactase activity of gnotobiotic piglets decreased in the weeks 4 ($p < 0.001$) and 5 ($p < 0.001$) to levels similar to those noticed at birth. Similarly, we recorded a post-weaning decrease in lactase specific activity also in conventional piglets. The results of these studies have been used to interpret the digestive and absorptive capacity of the small intestine as well as the maturity of the enterocytes.

5. Conclusion

Gnotobiotic animals are a very useful model in studying the physiology of the digestive tract. The gnotobiotic model allowed us to carry out systematic examination of the effect of a defined microbial population on postnatal intestinal development. We characterized regional variations in morphological and functional responses of the small intestine. We also identified that morphological and functional responses were affected differently by respective bacterial species, supporting the assumption that postnatal bacterial colonization patterns play an important role in neonatal intestinal development. Very good application of gnotobiotic animals is anticipated in the field of study of mutual interaction of natural microflora and pathogens in the digestive tract, mechanisms of probiotic effects of micro-organisms. We can conclude that the development of the intestinal mucosa membrane is in direct junction to breeding conditions. In connection with postnatal differentiation and the development of the small intestine in piglets, currently there is increasingly high interest in the explanation of the important role that can be played by colostrum and by milk containing growth factors, hormones and other bio-active compounds. It is likely that removal of milk will have a profound influence upon the processes regulating the growth of cells in the small intestine, their differentiation and function.

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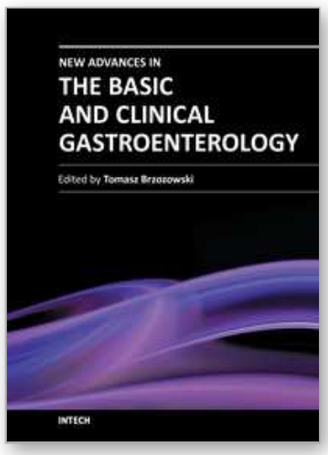

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The purpose of this book was to present the integrative, basic and clinical approaches based on recent developments in the field of gastroenterology. The most important advances in the pathophysiology and treatment of gastrointestinal disorders are discussed including; gastroesophageal reflux disease (GERD), peptic ulcer disease, irritable bowel disease (IBD), NSAIDs-induced gastroenteropathy and pancreatitis. Special focus was addressed to microbial aspects in the gut including recent achievements in the understanding of function of probiotic bacteria, their interaction with gastrointestinal epithelium and usefulness in the treatment of human disorders. We hope that this book will provide relevant new information useful to clinicians and basic scientists as well as to medical students, all looking for new advancements in the field of gastroenterology.

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