In vitro fermentation pattern in the large intestine of hybrids between wild boars and domestic pigs – a preliminary study

D. Miśta[1], B. Króliczewska[1], E. Pecka-Kiełb[1], J. Bujok[1], W. Zawadzki[1], J. Górecka[2], J. Piekarska[3]

[1]Department of Animal Physiology and Biostructure, Wroclaw University of Environmental and Life Sciences, Wrocław, Poland
[2]Department of Animal Products Technology and Quality Management, Wroclaw University of Environmental and Life Sciences, Wrocław, Poland
[3]Department of Internal Medicine and Clinic of Diseases of Horses, Dogs and Cats, Wroclaw University of Environmental and Life Sciences, Wrocław, Poland

ABSTRACT: Breeding of hybrids between wild boars and domestic pigs is in the consumer interest because of the need to ensure food security and diversification via widening the genetic basis of animals reared for meat. To expand the knowledge about their nutritional requirements, this study aimed to investigate hindgut fermentation in these animals. Caecal and colon cultures were incubated for 12 h *in vitro* with or without wheat bran as a supplementary substrate. Short-chain fatty acids, ammonia, methane, and total gas production were determined. The total concentrations of short-chain fatty acids in unincubated caecal and colon samples were 93.1 and 115 mmol/kg, respectively. The short-chain fatty acid profile in fresh hindgut contents was characterized by a high molar proportion of acetate (74.8–75.0 mol%), followed by propionate (18.2–18.5 mol%) and butyrate (5.4–5.5 mol%). The presence of wheat bran lowered acetate and increased butyrate, propionate, and valerate molar proportions. The ammonia level remained low (1.3–2.43 mmol/kg) regardless of the addition of the substrate. The relatively low pH and ammonia concentration in wild boar/pig hybrids may be caused by the low level of crude protein in diet of these animals. The rate of methanogenesis increased during the fermentation simultaneously with an increase in the production of gases after wheat bran addition. Methane production in the caecal and colon samples incubated with the substrate reached 15.6 and 16.1 mmol/kg, respectively. The hindgut fermentation pattern in wild boar/pig hybrids generally resembled that described earlier in domestic pigs, although some observed dissimilarities may be caused by distinct microbial activity.

Keywords: cross-breed; *Sus scrofa*; caecal fermentation; colon fermentation; short-chain fatty acids; methane

INTRODUCTION

Currently various actions are undertaken to promote genetic diversity in animal production. Their aim is to ensure food security and productivity, as well as animal resilience to biotic and abiotic threats in the environment. One of the new challenges is the need to increase the qual-

ity of products and food diversification to satisfy consumer requirements. Widening the genetic basis, within and between species of animals, is therefore essential. An important source of genetic variation may be breeding of hybrids between wild and domestic animals (Razmaite et al. 2009), which is becoming increasingly common in many countries. For example, hybrids between wild

boars (*Sus scrofa scrofa*) and domestic pigs (*Sus scrofa domesticus*) are raised for meat production. The wild boar/pig hybrids (WBPHs) are similar to wild boars in appearance and more resistant to harmful environmental factors than pigs. The meat of these animals tastes like venison, contains more protein, and is leaner and darker than pork (Klimas and Klimiene 2010). The growing interest in these hybrids has inspired scientists to take up research on physiology of these animals. Since WBPHs have different eating habits and diets than pigs, studies concerning digestive physiology, including hindgut microbial processes, are needed. Extending the knowledge of digestive physiology of the crossbreds can help us ensure nutritionally-adequate conditions for breeding of these animals and thus increase their productivity.

Microbial fermentation in the gastrointestinal tract of animals is a species-specific physiological adaptation and depends primarily on the size of the animal and its food habits (Pagan 2011). In non-ruminants, the caecum and colon are the most important chambers, where structural carbohydrates, which are not digested in the animal stomach and small intestine, are fermented by the local microflora. Bacterial density in the large intestine of pigs exceeds 10^9/g of digesta, which indicates a high fermentation capacity (Conway 1994). The concentration of end products of bacterial fermentation – such as short-chain fatty acids (SCFAs), ammonia, and gases (especially methane) – reflects the activity of the intestinal microflora, which promotes proper digestion.

Similarly to pigs and wild boars, WBPHs are hindgut fermenters, in which the microbial processes occur in the colon and caecum. These fermentation chambers are better developed in WBPHs than in pigs, which may lead to an assumption that microbial fermentation and fibrolytic activity are more intensive in the hybrids. The volume of the large intestine per 1 kg of metabolic body weight in WBPHs (75% of wild boar genes, 25% of Polish Large White pig genes) is more than twice as large as in fatteners (Polish Large White × Duroc × Hampshire pig) (Leroch et al. 2003). The lack of published data concerning microbial activity in the WBPH large intestine inspired us to undertake the present research, which may contribute to increased awareness of digestive physiology and nutritional requirements of these animals. The aim of this study was to investigate the pattern of microbial fermentation in the caecum and colon of WBPHs, based on microbial end-product analyses.

MATERIAL AND METHODS

Animals. The digestive tracts of nine WBPHs of the generation BC1 having 75% of wild boar blood and 25% of Pietrain pig blood were used in the study. These animals were reared outdoors, with a stocking rate of about 100 animals/ha, on a farm located in the Lubusz Land (West Poland). They were fed *ad libitum*, mainly with corn grain silage and meadow hay as well as oat grain twice a week (Table 1).

Two females and seven males aged about 1 year and weighing 30–45 kg were shot immediately before the experiment. Subsequently, the gastrointestinal tract of each animal was removed from the body, ligated and brought to the laboratory in pre-warmed isothermal containers. The caecum and colon were excised and slit, and digesta were used as inoculum for *in vitro* incubation, using CO_2 to obtain anaerobic conditions. In order to estimate animal health conditions, parasitological examinations of gastric and intestinal contents were also performed. Faecal egg or oocyst counts (nematodes and coccidia)

Table 1. Chemical composition of the wild boar/pig hybrid diet and the substrate used for *in vitro* fermentation

Item	Corn grain silage	Meadow hay	Oat grain	Wheat bran (substrate)
Dry matter (DM) (g/kg)	649	904	894	910
Crude protein (g/kg DM)	82.3	80.1	140	152
Ether extract (g/kg DM)	48.1	16.2	55.3	16.4
Crude fibre (g/kg DM)	34.4	320	144	56.4
Neutral detergent fibre (g/kg DM)	111	653	265	213
Acid detergent fibre (g/kg DM)	20.7	361	144	61.7
Ash (g/kg DM)	21.4	47.1	31.0	19.7
Non-structural carbohydrates (g/kg DM)	829	204	560	599
Gross energy (MJ/kg DM)	25.4	18.1	18.0	15.2

were generated using the concentration McMaster technique. The adult parasites were examined under a stereomicroscope and identified in accordance with the routine parasitological techniques.

In vitro fermentation model. *In vitro* caecal fermentation of the colon and caecal samples was performed to analyze methane production, SCFA and ammonia concentrations, and to measure the pH. Immediately after dissection, the colon and caecal contents were squeezed out and mixed with a spatula, and the pH was measured using a pH-meter CP-401 (Elmetron, Zabrze, Poland). Three 20 g samples were taken from each animal's colon or caecal content and transferred into 125 ml serum bottles. Each sample was diluted in 60 ml of buffer solution (pre-warmed to 39°C) containing per litre: 9.24 g of $NaHCO_3$, 2.75 g of $NaH_2PO_4 \cdot H_2O$, 470 mg of NaCl, 450 mg of KCl, 55 mg of $CaCl_2$, 72 mg of $MgCl_2 \cdot 6H_2O$, 22.2 mg of Na_2SO_4, 4.4 mg of $ZnSO_4 \cdot 7H_2O$, 1.2 mg of $CoCl_2 \cdot 6H_2O$, 19 mg of $MnSO_4 \cdot H_2O$, 36.8 mg of $FeSO_4 \cdot 7H_2O$, and 0.98 mg of $CuSO_4 \cdot 5H_2O$ (Adjiri et al. 1992). Two samples from each gut part were prepared for the incubation analyses, and the third sample was not incubated but immediately prepared for SCFA and ammonia analyses (B – blank samples). The incubation was performed without (C – control samples) or with the added substrate (1 g of wheat bran; S – samples with substrate). Before incubation, each serum bottle was thoroughly flushed with CO_2 to obtain anaerobic conditions and was then hermetically sealed with an isoprene rubber stopper and an aluminium cap, using a manual crimper. The incubation was performed in a shaking water bath at 39°C for 12 h.

Methane and total gas production. At the end of incubation, the headspace pressure inside each bottle was measured using a manual pressure manometer connected with a needle which was used to punch the rubber stopper on the serum bottle. The total gas production was calculated using the *C*lapeyron equation (ideal gas law), based on the gas pressure, the head-space volume, and the temperature inside the serum bottle*:*

$$PV = nRT \ (n = PV/RT)$$

where:
P = gas pressure
V = gas volume
n = number of moles of gas
R = universal gas constant (8.3145 J/mol K)
T = gas temperature

After measurement of the pressure, the fermentation gas was sampled with a gas-tight syringe for methane analysis by using the 7890A gas chromatograph (Agilent Technologies, Santa Clara, USA) equipped with a flame ionisation detector, thermal conductivity detector, two Supelco columns Porapak Q and HayeSep Q (Supelco, Bellefonte, USA), as well as a 5A Molecular Sieve. Helium was used as the carrier gas (flow rate: 25 ml/min). A standard curve for methane was prepared using gas mixtures certified for analyses (Linde; Agilent Technologies). Based on the gas pressure and the percentage of methane from the total gas volume, the molar concentration of methane was calculated using the Clapeyron equation.

Short-chain fatty acids analysis. In the remaining liquid samples, the fermentation was stopped by adding 0.05 ml of formic acid per 1 ml of the sample. The samples were centrifuged (2800 *g* for 20 min) and analyzed on a 7890A gas chromatograph (Agilent Technologies) with a flame ionization detector and Agilent J&W column DB-23, with helium as the carrier gas (flow rate 25 ml/min). This analysis was performed to determine the total SCFA concentration and the molar proportions of each fatty acid (acetate, propionate, isobutyrate, butyrate, isovalerate, valerate, and caproate). Standard curves were prepared using the Volatile Free Acid Mix (Supelco) and the concentration of each acid was calculated. 1,3-Propanediol was used as an internal standard for SCFAs.

Ammonia and pH measurements. Ammonia in all diluted samples was separated by microdiffusion in Conway units, and the concentration was determined with a Nessler reagent by using a Lambda XLS spectrophotometer (PerkinElmer Inc., Waltham, USA) at a wavelength of 410 nm. Also pH values were measured by using a pH-meter CP-401 (Elmetron) in all liquid samples.

Chemical analyses. The main ingredients of the WBPH diet, and a sample of wheat bran used as the substrate for *in vitro* fermentation were analyzed using standard methods: dry matter (DM; method 934.01 of AOAC 2005), crude protein (CP; Kjeldahl method, method 984.13 of AOAC 2005), ether extract (EE; method 920.39 of AOAC 2005), crude fibre (CF; method 978.10 of AOAC 2005), ash (method 942.05 of AOAC 2005), neutral detergent fibre (NDF; method of Holst 1973), and acid detergent fibre (ADF; method 973.18 of AOAC 2005). The gross energy in the samples was measured using an

Czech J. Anim. Sci., 61, 2016 (11): 506–514

Original Paper

adiabatic oxygen bomb calorimeter KL-10 (Precyzja, Bydgoszcz, Poland). The content of non-structural carbohydrates (NSC) was calculated according to National Research Council (2001):

1000 – (ash + CP + EE + NDF)

where ash, crude protein (CP), ether extract (EE), and neutral detergent fibre (NDF) contents were expressed as g/kg of DM. Chemical composition of the main diet ingredients and the substrate is displayed in Table 1.

Statistical analysis. The obtained data were subjected to one-way analysis of variance (ANOVA), using STATISTICA Version 10.0 for MS Windows software package to compare the *in vitro* fermentation parameters. We analyzed the effect of the gut part (caecum vs colon), substrate addition to the incubated samples (C vs S), and incubation time of C and S samples (B vs C and B vs S) on the fermentation parameters. Differences between means with $P < 0.05$ were accepted as statistically significant.

RESULTS AND DISCUSSION

The caecal and colon fermentation parameters, including sample pH, total SCFA concentration, SCFA molar proportions, and ammonia concentration are compared in Table 2. The total concentration of SCFAs reached 93.1 mmol/kg in the caecum and 115 mmol/kg in the colon.

The total concentration of SCFAs measured in the large intestine of the species studied so far fitted within the range of 30–240 mM, but most commonly averaged 70–120 mM (Bergman 1990; Mista et al. 2015; Pecka-Kielb et al. 2016). There are no published data concerning fermentation processes in WBPHs, so we compared our results to the values obtained for pigs, as the closest relatives of these animals. In the present study, the total SCFA concentration in the WBPH fresh hindgut contents was similar to that observed in pigs and ranged from 65.3 to 156 mmol/kg wet weight of caecal digesta (Marounek et al. 2002; Van Nevel et

Table 2. Production of short-chain fatty acids (SCFAs), ammonia, and gases in wild boar/pig hybrid caecal and colon inocula during 12-hour *in vitro* fermentation

Fermentation parameter	Caecum			Colon			SEM	*P*-value[a]			
	B	C	S	B	C	S		substrate	gut part	incubation	
										B vs C	B vs S
Total SCFAs[b]	93.1	222	303	115	230	326	13.96	< 0.001	0.443	< 0.001	< 0.001
SCFAs[c]											
Acetate	75.0	69.4	64.2	74.8	68.0	64.0	0.818	0.003	0.620	< 0.001	< 0.001
Propionate	18.2	20.4	22.8	18.5	21.8	24.0	0.492	0.032	0.250	0.009	< 0.001
Isobutyrate	0.37	0.69	0.55	0.37	0.66	0.56	0.034	0.131	0.995	< 0.001	0.004
Butyrate	5.49	7.56	10.3	5.39	7.85	9.54	0.330	< 0.001	0.887	< 0.001	< 0.001
Isovalerate	0.28	1.05	1.00	0.38	1.03	0.86	0.065	0.415	0.964	< 0.001	< 0.001
Valerate	0.65	0.97	1.20	0.51	0.68	0.96	0.050	0.044	0.030	0.033	< 0.001
Caproate	nd	0.004	0.002	0.01	nd	0.002	0.001	0.922	0.878	0.881	0.796
SCFA ratios											
Acetate : propionate	4.21	3.46	2.87	4.17	3.22	2.73	0.118	0.007	0.484	0.001	< 0.001
Propionate : butyrate	3.54	2.78	2.28	3.54	2.85	2.60	0.111	0.058	0.626	0.006	< 0.001
Ammonia[b]	2.12	1.61	1.30	2.43	1.48	1.48	0.099	0.416	0.545	0.003	< 0.001
pH of fresh content	5.86	–	–	5.72	–	–	0.050	–	0.164	–	–
pH of diluted sample	7.24	6.57	6.06	7.09	6.45	6.09	0.066	< 0.001	0.564	< 0.001	< 0.001
Methane[b]	–	8.22	15.6	–	10.0	16.1	0.839	< 0.001	0.490	–	–
Gas production[b]	–	70.2	155	–	87.4	150	7.021	< 0.001	0.667	–	–

B = blank samples (unincubated), C = control samples (incubated without the substrate), S = samples incubated with the substrate, nd = not detected

[a]probability of effects of substrate (C vs S), gut part (caecum vs colon), and incubation time for C samples (B vs C) and for S samples (B vs S), [b]mmol/kg of digesta, [c]mol in 100 mol of total SCFAs (mol%)

Original Paper

Czech J. Anim. Sci., 61, 2016 (11): 506–514

al. 2006; Suarez-Belloch et al. 2013) and from 77.2 to 158 mmol/kg of wet weight of colon digesta (Loh et al. 2006; Van Nevel et al. 2006). Additionally, our results show SCFA concentrations similar to those in caecum and colon of wild boars (Pecka-Kielb et al. 2016).

Le Goff et al. (2003) found that the intrinsic capacity of intestinal flora to degrade dietary fibre is not involved in the improvement of dietary fibre digestibility in older pigs. The improved digestibility with an increased body weight may be explained by the longer retention time due to larger volume of the hindgut (Le Goff et al. 2003). That suggestion might lead to the assumption that the higher volume of the large intestine of WBPH, as compared to that of pigs (Leroch et al. 2003), may also improve its capacity for dietary fibre degradation, resulting in high SCFA production.

Wheat bran is a rich source of insoluble, slowly fermentable fibre (Table 1), which may enhance the microbial fermentation activity in the large intestine (Molist et al. 2009). In the present study, addition of wheat bran to the inocula resulted in raised SCFA concentrations after the 12-h incubation ($P < 0.001$, Table 2). The caecal and colon SCFA production was elevated also in pigs fed diets containing wheat bran as a fibre supplementation (Govers et al. 1999; Molist et al. 2009).

The SCFA profiles in fresh (unincubated) caecal and colon contents of WBPHs were similar and characterized by high molar proportions of acetate (74.8–75.0 mol%), followed by propionate (18.2–18.5 mol%) and butyrate (5.4–5.5 mol%) (Table 2). Only the valerate proportion was higher in the caecal than in the colon contents, but most of these values did not exceed 1 mol%. The ratios between the main SCFAs were also similar in the caecum and colon of WBPHs. The acetate : propionate ratio reached 4.2, while the propionate : butyrate ratio was 3.5 in unincubated samples and tended to decrease after the incubation. After the 12-h fermentation the mutual proportions of SCFAs changed and the effect of wheat bran addition on the SCFA profile was observed (Table 2). After the incubation, we observed a decrease in acetate molar proportions ($P < 0.001$) accompanied by an increase in propionate ($P < 0.01$) as well as isobutyrate, butyrate, isovalerate ($P < 0.001$), and valerate ($P < 0.05$) molar proportions in C samples. Similar, but sometimes stronger, effects concerning S samples were also noted. Incubation time

diminished the acetate : propionate and the propionate : butyrate ratios in both C ($P < 0.01$) and S samples ($P < 0.001$).

The molar ratio of acetate : propionate : butyrate in the large intestine of animals, such as horses, sheep, pigs, and various wild species, has been found to approximate 70 : 20 : 10 (Bergman 1990). This ratio in the fresh caecal and colon content of WBPHs was about 75 : 20 : 5 in the present study, so it differed from that obtained in pigs, whose intestinal microflora produces more propionate (about 30 mol%) and butyrate (about 10 mol%) (Marounek et al. 2002; Loh et al. 2006; Van Nevel et al. 2006). In fresh caecal and colon ingesta of wild boars, butyrate proportions were similar to those obtained in pigs, but propionate proportions were lower from pigs and WBPHs and reached 10–13 mol% (Pecka-Kielb et al. 2016). These differences may be caused by less abundant microorganisms with the potential to produce propionate in wild boar and WBPHs (and butyrate in WBPHs), but may also be the result of lower activity of these microorganisms caused by different diets. The profile of SCFAs produced by caecal and colon microbiota depends largely on the composition of food. Diets with high proportion of concentrates, rich in readily fermented non-structural carbohydrates, allow for faster fermentation with the elevated production of propionate, which decreases the acetate : propionate ratios in the digestive tract (Soren et al. 2015).

Some authors found that the source of fibre in diets (oat bran or wheat bran) influences acetic and butyric acid proportions (Christensen et al. 1999). In the present study, the addition of wheat bran lowered the molar proportion of acetate ($P < 0.01$) and increased butyrate ($P < 0.001$), as well as propionate and valerate ($P < 0.05$) molar proportions, but did not affect iso-acids. A decreasing effect of substrate addition on acetate : propionate ratio was also noted ($P < 0.01$).

There are studies showing that wheat bran dietary supplementation increased butyrate proportion in the colon of pigs because of the influence of insoluble polysaccharides on resistant starch fermentation (Govers et al. 1999). According to Molist et al. (2009), diet containing a large amount of insoluble non-structural polysaccharides promoted a beneficial shift in the microbial colonization in the large intestine, with increase in butyrate production and decrease in enterobacteria counts.

Czech J. Anim. Sci., 61, 2016 (11): 506–514

Original Paper

Furthermore, the probable reason of the drop in acetate : propionate ratio in our experiment is the enhanced supply of carbohydrates with the substrate added to the incubated samples.

Other SCFAs, such as isobutyric, valeric, isovaleric, and caproic acids, were present in the pig hindgut in smaller amounts. Their molar proportions reported by O'Shea et al. (2011) in the pig caecum were similar to WBPH results obtained in our research. However, the results presented by those authors for colon contents were slightly higher: isobutyrate 1.1–1.4 mmol/kg, isovalerate 1.3–1.8 mmol/kg, and valerate 1.2–1.7 mmol/kg (O'Shea et al. 2011). Branched-chain fatty acids (BCFAs) derive from bacterial proteolysis, particularly from fermentation of branched amino acids and, subsequently, they are utilized by cellulolytic bacteria for their proliferation (Marounek et al. 2002). An increase in BCFAs tends to be observed when carbohydrates are limited, as is the case with increased ammonia production (Casadei et al. 2009).

Since nutritional requirements of crude protein for pigs are about 190–200 g/kg DM (Kaczmarek and Rzasa 2005; Suarez Belloch et al. 2013), it is obvious that our WBPHs received relatively low amount of CP (Table 1). It might improve the gut health because high level of dietary protein predisposes pig intestine to the condition conductive to *Escherichia coli* colonization (Jha and Berrocoso 2016). Moreover, the decrease in protein consumption causes increase in carbohydrate : protein ratio entering the pig's large intestine beneficially influencing microbial fermentation in large intestine (Pieper et al. 2008). Lowering the amount of CP in pig nutrition may lead to minimizing odour of manure via reduction of bacterial proteolysis which induces production of BCFAs, ammonia, amines, and indolic, phenolic, and sulphurous compounds in the gut (Jha and Berrocoso 2016). It can be assumed that low requirements for protein in WBPHs diet could result in lower odour emission accompanying rearing of these animals in comparison to fattener breeding.

In the present study, the ammonia concentration did not change significantly in the examined gut parts of WBPHs. In fresh caecal content it was 2.12 mmol/kg compared to 2.43 mmol/kg in colon (Table 2). The wheat bran addition to caecal and colon inocula did not influence the ammonia production in our *in vitro* experiment, but its concentration diminished during incubation (Table 2).

The wheat bran added to our samples of inocula contained higher CP level than the WBPH diet. Oat grain, which is relatively rich in protein, was fed to animals only twice a week (neither on the day of animal slaughter, nor the day before). Surprisingly, despite its content of protein, the wheat bran substrate did not affect the ammonia production. This result can be explained by reducing effect of fermentable carbohydrates on protein fermentation in large intestine (Bikker et al. 2006). Insoluble fibre sources (e.g. wheat bran) cause prolonged bacterial fermentation and limit the occurrence of proteolytic fermentation in the large intestine (Shim et al. 2007). Dietary wheat bran inclusion also decreased microbial fermentation of proteins in pig digestive tract (Govers et al. 1999). The 12-hour *in vitro* incubation of wild boar caecal and colon inocula resulted in even lower ammonia production compared to WBPHs, which also remained unaffected by the wheat bran addition (Pecka-Kielb et al. 2016).

The caecal and colon ammonia concentrations of WBPHs obtained in the present study were lower than those observed by other researchers in pigs (Govers et al. 1999; Marounek et al. 2002; Van Nevel et al. 2006). According to Marounek et al. (2002), ammonia concentration in fresh caecal digesta in pigs is about 15 mmol/kg, however, Govers et al. (1999) reported considerably higher concentrations, ranging from about 25 to 40 mmol/kg. In the present study, the low ammonia level, together with slightly lower proportions of BCFAs in WBPH than in pig colon contents, could be due to lower crude protein level in the WBPH diet.

Reduction of ammonia emission in pigs fed diets high in fermentable dietary fibre is accompanied by lowering pH of faeces and manure (Aarnink and Verstegen 2007). Also, according to Jensen and Jorgensen (1994), pH in the pig caecum and colon depends on fibre content in the diet. According to those authors, a high-fibre diet resulted in a decrease in hindgut pH, which was 5.6 in caecal and 6.0 in colon samples. Conversely, low-fibre diet raised caecal and colon pH, which was 6.3 and 6.5, respectively (Jensen and Jorgensen 1994).

The pH values of WBPHs were similar in the caecum and the colon, and in fresh, undiluted material achieved mean values of 5.86 and 5.72, respectively (Table 2). We can suppose that slightly lower pH of the fresh large intestinal samples of WBPHs in the present study, as compared to aver-

Original Paper

Czech J. Anim. Sci., 61, 2016 (11): 506–514

age values obtained for pigs by other authors, may be caused by the higher contribution of fibre in the WBPH diet or by higher fibrolytic activity in the WPBH large intestine. The inoculum reaction of caecal and colon samples significantly decreased as a result of prolonged incubation time, as well as of bran addition ($P < 0.001$). Consequently, pH values decreased with increasing SCFA levels.

The presence of wheat bran was responsible for twice as high production of gases during the microbial fermentation ($P < 0.001$) (Table 2). The methanogenesis increased simultaneously with the gas production ($P < 0.001$) and reached 8.22 mmol/kg in caecal control samples and 15.6 mmol/kg in the samples incubated with wheat bran addition, compared to 10.0 and 16.1 mmol/kg in the colon, respectively.

Gas production variables were recorded as indicative of intestinal microbial energetic metabolism (Casadei et al. 2009). In our experiment, the addition of the substrate to the incubated *in vitro* samples enhanced the supply of energy and, consequently, enlarged the production of gases resulting from an increase in microbial fermentation intensity. Methanogenesis in the animal large intestine is a consequence of a series of metabolic interactions among various groups of microorganisms. Previous studies have indicated a competition for metabolic hydrogen uptake between the methanogenic Archaea and acetogenic bacteria in the animal digestive tract, which leads to an increase in methane production with age (De Graeve et al. 1994). Methane is a very potent greenhouse gas and its emission in piglets ranges from 0.11 to 0.15 l/day, in growing pigs 0.28–12.1 l/day, and in adult sows 3.2–28.7 l/day (Jorgensen et al. 2011). It is largely dependent on diet, especially on the level of fermentable fibre, which increases the excretion of methane. The rate of methanogenesis was higher in the pig colon than in the caecum and grew when pigs were fed high-fibre diets (Jensen and Jorgensen 1994). In our study, the methanogenesis in WBPHs increased after the addition of wheat bran. Methane production after 8 h of the *in vitro* incubation of pig caecal contents with starch amounted to 6.14 mmol/l (Marounek et al. 1997). The rate of methanogenesis in WBPH caecal inocula appeared higher in our experiment, but this may be caused by longer incubation time. It seems to be closer to pig's than wild boar's methane production which was lower

and did not exceed 5 mmol/kg of intestinal content (Pecka-Kielb et al. 2016).

In parasitological examinations, eggs of gastrointestinal nematodes, lungworms and oocysts of coccidia were found in the faecal samples. The most extensive infection was recorded for *Oesophagostomum* spp. (100%) and *Eimeria* spp. (89%), the common parasites of pigs kept under various management systems (free-range, organic, and conventional farms) (Eijck and Borgsteede 2005; Jankowska-Makosa and Knecht 2015). Also typical wild boar nematodes were detected: *Ascarops strongylina*, *Globocephalus* sp. (33%), *Physocephalus sexalatus*, *Strongyloides ransomi*, *Trichuris suis* (22%), and *Metastrongylus* spp. (10%). The intensity of parasite infections was not high despite free access to intermediate hosts and dispersal form of parasites in the soil. The faecal egg intensities, determined as mean egg count/g of faeces were: *Eimeria* spp. 1675, *Oesophagostomum* spp. 730, *Globocephalus* spp. 467, *Physocephalus sexalatus* 225, *Metastrongylus* spp. 175, *Ascarops strongylina* 133, *Strongyloides ransomi* and *Trichuris suis* 75. It is possible that microbial fermentation occurring in the large intestine of WBPHs limited gastrointestinal parasites' development via the production of SCFAs which, by changing the expression of chemokines in enterocytes, play a role in the antiparasitic response (Sanderson 2004). Their antiparasite effect on the course of intestinal invasion of *Ascaris suum*, *Oesophagostomum dentatum*, and *Trichuris suis* was observed (Varadyova et al. 2001; Petkevicius et al. 2003, 2007).

CONCLUSION

The analyses of caecal and colon microbial metabolites showed that the molar ratio of acetate : propionate : butyrate in WBPHs ranged from about 75 : 20 : 5 in unincubated samples to 65 : 25 : 10 in the samples incubated with the substrate (wheat bran). Moreover, the WBPH fermentation profile was characterized by relatively low pH and ammonia concentration, which may be caused by the low level of CP in their diet. However, high fibrolytic and/or low proteolytic activity in the WPBH large intestine may also induce these results. Further microbiological examinations concerning the WBPH gastrointestinal tract as well as comparative studies of WBPHs and pigs could improve the understanding of digestive physiology of WBPHs.

Czech J. Anim. Sci., 61, 2016 (11): 506–514

Original Paper

REFERENCES

Aarnink A.J.A., Verstegen M.W.A. (2007): Nutrition, key factor to reduce environmental load from pig production. Livestock Science, 109, 194–203.

Adjiri D., Bouilier-Oudot M., Lebas F., Candau M. (1992): In vitro simulation of rabbit caecal fermentation in a semi-continuous flow fermentor. I. Effect of food substrate pretreatment. Reproduction Nutrition Development, 32, 351–360.

AOAC (2005): Official Methods of Analysis of AOAC International. 18th Ed. Association of Official Analytical Chemists, Arlington, USA.

Bergman E.N. (1990): Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. Physiology Review, 70, 567–590.

Bikker P., Dirkzwager A., Fledderus J., Trevisi P., Huerou-Luron I.L., Lalles J.P., Awati A. (2006): The effect of dietary protein and fermentable carbohydrates levels on growth performance and intestinal characteristics in newly weaned piglets. Journal of Animal Science, 84, 3337–3345.

Casadei G., Grilli E., Piva A. (2009): Pediocin A modulates intestinal microflora metabolism in swine in vitro intestinal fermentations. Journal of Animal Science, 87, 2020–2028.

Christensen D.N., Bach Knudsen K.E., Wolstrup J., Jensen B.B. (1999): Integration of ileum cannulated pigs and in vitro fermentation to quantify the effect of diet composition on the amount of short-chain fatty acids available from fermentation in the large intestine. Journal of the Science of Food and Agriculture, 79, 755–762.

Conway P.L. (1994): Function and regulation of the gastrointestinal microbiota of the pig. In: Souffrant W.B., Hagemeister H. (eds): Proc. 6th Internat. Symposium on Digestive Physiology in Pigs, Dummerstorf, Germany, 231–240.

De Graeve K.G., Grivet J.P., Durand M., Beaumatin P., Cordelet C., Hannequart G., Demeyer D. (1994): Competition between reductive acetogenesis and methanogenesis in the pig large-intestinal flora. Journal of Applied Bacteriology, 76, 55–61.

Eijck I.A., Borgsteede F.H. (2005): A survey of gastrointestinal pig parasites on free-range, organic and conventional pig farms in the Netherlands. Veterinary Research Communications, 29, 407–414.

Govers M.J.A.P., Gannon N.J., Dunshea F.R., Gibson P.R., Muir J.G. (1999): Wheat bran affects the site of fermentation of resistant starch and luminal indexes related to colon cancer risk: a study in pigs. Gut, 45, 840–847.

Holst D.O. (1973): Holst filtration apparatus for Van Soest detergent fiber analysis. Journal of AOAC International, 56, 1352–1356.

Jankowska-Makosa A., Knecht D. (2015): The influence of endoparasites on selected production parameters in pigs in various housing systems. Research in Veterinary Science, 100, 153–160.

Jensen B.B., Jorgensen H. (1994): Effect of dietary fiber on microbial activity and microbial gas production in various regions of the gastrointestinal tract of pigs. Applied and Environmental Microbiology, 60, 1897–1904.

Jha R., Berrocoso J.F.D. (2016): Dietary fiber and protein fermentation in the intestine of swine and their interactive effects on gut health and on the environment: A review. Animal Feed Science and Technology, 212, 18–26.

Jorgensen H., Theil P.K., Bach Knudsen K.E. (2011): Enteric methane emission from pigs. In: Carayannis E.G. (ed.): Planet Earth 2011 – Global Warming Challenges and Opportunities. InTech, Rijeka, 605–622.

Kaczmarek P., Rzasa A. (2005): Effect of diet containing fish meal on physicochemical and organoleptical properties of fatteners meat. Roczniki Naukowe Polskiego Towarzystwa Zootechnicznego, 1, 359–366. (in Polish)

Klimas R., Klimiene A. (2010): Some biological characteristics of hybrids (Sus domesticus × Sus scrofa L.). Acta Biologica Universitatis Daugavpiliensis, 10, 31–36.

Le Goff G., Noblet J., Cherbut C. (2003): Intrinsic ability of the faecal microbial flora to ferment dietary fibre at different growth stages of pigs. Livestock Production Science, 81, 75–87.

Leroch R., Fuchs B., Szuba-Trznadel A. (2003): Comparison of the digestive tracts in swine, wild boars and in the swine × boars hybrids. Acta Scientiarum Polonorum Zootechnica, 2, 47–54.

Loh G., Eberhard M., Brunner R.M., Hennig U., Kuhla S., Kleessen B., Metges C.C. (2006): Inulin alters the intestinal microbiota and short-chain fatty acid concentrations in growing pigs regardless of their basal diet. Journal of Nutrition, 136, 1198–1202.

Marounek M., Savka O.G., Skrivanova V. (1997): Effect of salinomycin on in vitro caecal fermentation in pigs. Journal of Animal Physiology and Animal Nutrition, 77, 111–116.

Marounek M., Adamec T., Skrivanova V., Latsik N.I. (2002): Nitrogen and in vitro fermentation of nitrogenous substrates in caecal contents of the pig. Acta Veterinaria Brno, 71, 429–433.

Mista D., Kroliczewska B., Marounek M., Pecka E., Zawadzki W., Nicpon J. (2015): In vitro study and comparison of caecal methanogenesis and fermentation pattern in

Original Paper

Czech J. Anim. Sci., 61, 2016 (11): 506–514

the brown hare (Lepus europaeus) and domestic rabbit (Oryctolagus cuniculus). PLoS ONE, 10, e0117117.

Molist F., Gomez de Segura A., Gasa J., Hermes R.G., Manzanilla E.G., Anguita M., Perez J.F. (2009): Effects of the insoluble and soluble dietary fibre on the physicochemical properties of digesta and the microbial activity in early weaned piglets. Animal Feed Science and Technology, 149, 346–353.

National Research Council (2001): Nutrient Requirements of Dairy Cattle. 7th Ed. The National Academies Press, Washington, DC, USA.

O'Shea C.J., Sweeney T., Lynch M.B., Callan J.J., O'Doherty J.V. (2011): Modification of selected bacteria and markers of protein fermentation in the distal gastrointestinal tract of pigs upon consumption of chitosan is accompanied by heightened manure odor emissions. Journal of Animal Science, 89, 1366–1375.

Pagan J.D. (2011): Fermentation key for wide range of species. Feedstuffs, 83, 10(2).

Pecka-Kielb E., Bujok J., Mista D., Kroliczewska B., Gorecka J., Zawadzki W. (2016): In vitro study of caecal and colon microbial fermentation patterns in wild boar (Sus scrofa scrofa). Folia Biologica (Krakow), 64, 31–36.

Petkevicius S., Bach Knudsen K.E., Murrell K.D. (2003): Effects of Oesophagostomum dentatum and dietary carbohydrates on morphology of the large intestine of pigs. Veterinary Parasitology, 116, 125–138.

Petkevicius S., Thomsen L.E., Bach Knudsen K.E., Murrell K.D., Roepstorff A., Boes J. (2007): The effect of inulin on new and on patent infections of Trichuris suis in growing pigs. Parasitology, 134, 121–127.

Pieper R., Jha R., Rossnagel B., Van Kessel A.G., Souffrant W.B., Leterme P. (2008): Effect of barley and oat cultivars with different carbohydrate compositions on the intestinal bacterial communities in weaned piglets. FEMS Microbiology Ecology, 66, 556–566.

Razmaite V., Kerziene S., Jatkauskiene V. (2009): Body and carcass measurements and organ weights of Lithuanian indigenous pigs and their wild boar hybrids. Animal Science Papers and Reports, 27, 331–342.

Sanderson I.R. (2004): Short chain fatty acid regulation of signaling genes expressed by the intestinal epithelium. The Journal of Nutrition, 134, 2450–2454.

Shim S.B., Verdonk J.M.A.J., Pellikaan W.F., Verstegen M.W.A. (2007): Differences in microbial activities of faeces from weaned and unweaned pigs in relation to in vitro fermentation of different sources of inulin-type oligofructose and pig feed ingredients. Asian-Australasian Journal of Animal Sciences, 20, 1444–1452.

Soren N.M., Sejian V., Malik P.K. (2015): Enteric methane emission under different feeding systems. In: Sejian V., Gaughan J., Baumgard L., Prasad C. (eds): Climate Change Impact on Livestock: Adaptation and Mitigation. Springer (India) Private Ltd, New Delhi, 187–208.

Suarez-Belloch J., Doti S., Rodriguez-Romero N., Guada J.A., Fondevila M., Latorre M.A. (2013): Hindgut fermentation in pigs induced by diets with different sources or starch. Spanish Journal of Agricultural Research, 11, 780–789.

Van Nevel J.C., Dierick N.A., Decuypere J.A., Smet S. (2006): In vitro fermentability and physicochemical properties of fibre substrates and their effect on bacteriological and morphological characteristics of the gastrointestinal tract of newly weaned piglets. Archives of Animal Nutrition, 60, 477–500.

Varadyova Z., Zelenak I., Siroka P., Dubinsky P. (2001): In vitro fermentation of celulosis amorphous and meadow hay in experimentally Ascaris suum-infected lambs. Small Ruminant Research, 40, 155–164.

Corresponding Author

Dr. Dorota Miśta, DVM, Ph.D., Wroclaw University of Environmental and Life Sciences,
Department of Animal Physiology and Biostructure, Norwida 31, 50-375 Wrocław, Poland
Phone: +48 713 205 437, e-mail: dorota.mista@up.wroc.pl