Combinations of feed additives affect ileal fibre digestibility and bacterial numbers in ileal digesta of piglets

E. Weiss[1], M. Eklund[1], A. Semaskaite[2], R. Urbaityte[1], B. Metzler-Zebeli[3], N. Sauer[1], A. Ratriyanto[4], R. Gruzauskas[2], R. Mosenthin[1]

[1]Institute of Animal Nutrition, University of Hohenheim, Stuttgart, Germany
[2]Veterinary Academy, Lithuanian University of Health Sciences, Kaunas, Lithuania
[3]Clinic for Swine, Department for Farm Animals and Veterinary Public Health, University of Veterinary Medicine, Vienna, Austria
[4]Department of Animal Science, Sebelas Maret University, Surakarta, Indonesia

ABSTRACT: The study was performed to investigate the effect of combinations of feed additives consisting of either a combination of a probiotic and a prebiotic (synbiotic), or a combination of a probiotic and xylanase on ileal nutrient digestibility, ileal microbial metabolite concentrations, and microbial composition in ileal digesta of weaned piglets. In total, 12 six-week old barrows with an average initial body weight of 7.5 kg, fitted with an ileal T-cannula, were assigned to 3 dietary treatments. The basal diet was supplemented with a combination of 1 g probiotics (*Pediococcus acidilactici,* Bactocell®) and 20 g prebiotics (oligofructose) (Pro/Pre) or 1 g probiotics (*Pediococcus acidilactici,* Bactocell®) and 0.5 g xylanase (Pro/Xyl) per kg diet. The supplementation of Pro/Xyl increased ileal digestibility of neutral detergent fibre (NDF) by 41.6% ($P < 0.05$). The microbial metabolite concentrations, pH of ileal digesta as well as ileal lactobacilli populations were not affected ($P > 0.05$) by any of the treatments. *Enterobacteriaceae* were reduced ($P < 0.05$) upon supplementation of Pro/Pre, resulting in an increased ($P < 0.05$) ileal lactobacilli to *Enterobacteriaceae* ratio compared with piglets fed the control diet. In conclusion, NDF digestibility was increased following xylanase supplementation, while microbial activity and composition in the ileum were not affected. The synbiotic approach was effective in reducing ileal *Enterobacteriaceae* numbers.

Keywords: synbiotic; feed enzyme; intestine; short-chain fatty acid; microbiota; pig

Previous research has shown that a well-balanced intestinal microbiota may support healthy animals in efficient digestion and maximum uptake of nutrients, as well as in increasing the body's resistance to infections, thereby protecting the host against enteropathic diseases (Kyriakis et al., 1999; Rolfe, 2000). Dietary inclusion of feed additives such as probiotics and prebiotics has been suggested to stimulate proliferation and metabolic activity of beneficial bacteria such as bifidobacteria and lactobacilli (Metzler et al., 2005). Probiotics are defined as live microorganisms that benefit the host by e.g. maintaining the intestinal ecosystem, improving animal health, and increasing nutrient digestibility and growth performance (Fuller, 1989; De Lange et al., 2010). A prebiotic is defined as a selectively fermented ingredient that results in specific changes, both in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefit upon host health (Gibson et al., 2010). The combination of probiotics and prebiotics, also referred to as synbiotics, suggests associate effects, mediated by an increased survival/activity of the probiotic in the host's gastrointestinal tract (GIT), while also stimulating indigenous beneficial bacteria (Roberfroid, 1998).

Supplementation of exogenous enzymes to pigs' diets has been proven to be an alternative strategy to improve animal performance by reducing or eliminating anti-nutritive factors present in feedstuffs, thereby increasing nutrient digestibility and

availability (De Lange et al., 2010). For example, supplemental xylanase has been shown to improve the nutritional value of diets with high amounts of non-starch-polysaccharides (NSP), by partially hydrolyzing soluble and insoluble NSP (Simon, 1998; Diebold et al., 2004). In addition, it has been suggested that hydrolysis of polysaccharide results in the generation of oligosaccharides containing e.g. xylose or arabinose that might be utilized as prebiotic substrates for bacterial fermentation (Pluske et al., 2002; Kiarie et al., 2007). There is evidence that such prebiotic substances favour the proliferation of lactic acid bacteria (Högberg and Lindberg, 2004; Kiarie et al., 2007), thereby inhibiting growth of pathogens e.g. due to competitive exclusion (Hillman et al., 1995).

A combination of different feed additives rather than the use of individual feed additives has been suggested to be more effective in livestock feeding (De Lange et al., 2010). For probiotics, it has been hypothesized that their efficacy may be improved by concomitant delivering their appropriate substrates, i.e. either by direct supplementation with a prebiotic, or, indirectly, by supplementing an enzyme generating prebiotic substances. However, at the ileal level, only few studies with weaned pigs focussing on potential associated effects of pro- and prebiotics both on nutrient digestibility and on microbiota composition including its metabolic activity have been performed, but, to our knowledge, there are no studies in which the combination of probiotics and xylanase has been assessed. As for the pig substantial microbial activity has been shown for the proximal GIT (Houdijk et al., 2002), the present study with weaned pigs aimed to determine the impact of combinations of supplemental feed additives on ileal nutrient digestibility and ileal numbers of marker bacteria such as *Lactobacillus* spp. and *Enterobacteriaceae*, and microbial metabolic activity.

MATERIAL AND METHODS

Animals and housing

The experiment was performed with 12 six-week-old barrows (German Landrace × Piétrain) with an average initial and final body weight (BW) of 7.5 ± 0.49 kg and 8.2 ± 0.57 kg, respectively. The pigs were fitted with simple T-cannulas at the distal ileum according to the principles described by Li et al. (1993). They were kept individually in stainless steel metabolic crates. The research unit was equipped with an automated temperature control system kept at 23°C. Each metabolic crate was equipped with an infrared heating lamp and a low pressure drinking nipple. The research protocol was approved by the German Ethical Commission for Animal Welfare. Care of the animals used in this experiment was in accordance with the corresponding Council Directive (1986).

Experimental design and dietary treatments

The 12 animals were completely randomized to either the control diet or to one of the two experimental diets resulting in four observations per treatment. They were fed a basal diet consisting mainly of wheat, barley, and soybean meal. Diet formulation of the basal diet is summarized in Table 1. The feedstuffs were ground to pass a 2 mm mesh screen prior to mixing of the diets. The basal diet was formulated to meet or to exceed the nutrient requirements of the National Research Council (1998) for piglets from 5 to 10 kg BW. The diet was free of antibiotics to prevent possible impacts on the composition and activity of the intestinal microbiota. Titanium dioxide (TiO_2) (5 g/kg) was used as a digestibility marker. The basal diet was fed alone (control treatment (Con)) or supplemented with combinations of 1 g probiotics and 20 g prebiotics (Pro/Pre), or with 1 g probiotics and 0.5 g xylanase (Pro/Xyl) per kg diet. The probiotic preparation used in the present study contained *Pediococcus acidilactici* MA18/5M (Bactocell®, 1.0×10^{10} CFU/g) (Lallemand Animal Nutrition SA, Blagnac, France). The prebiotic preparation consisted mainly of oligofructose (≥ 93%) (Raftifeed® OPS; BENEO-Orafti, Tienen, Belgium), produced by partial hydrolysis of chicory inulin. The inclusion levels for the pre- and probiotics used in this study were in the range of previously reported dosages in diets for pigs. A preparation of an endo-1,4-β-xylanase (EC 3.2.1.8, Natugrain® Wheat TS) (BASF AG, Ludwigshafen, Germany) with a minimum activity of 5600 TXU/g was added according to recommendations of the manufacturer. One TXU is defined as the amount of enzyme which liberates 5μM of reducing sugars (xylose equivalents) from wheat arabinoxylan per min at pH 3.5 and 55°C. The supplements were added to the basal diet at the expense of corn

Table 1. Formulation of the basal diet (g/kg, as fed)

Ingredient	Basal
Wheat	490.0
Soybean meal	230.0
Barley	158.2
Wheat bran	50.0
Soybean oil	20.0
Corn starch	10.0
Sodium chloride	1.3
L-Lysine-HCl	0.5
Mineral and vitamin premix*	35.0
Titanium dioxide	5.0

*Vitamiral FE (Agravis Futtermittel GmbH, Münster, Germany) supplied the following (per kg diet): calcium 7.8 g, phosphorus 2.8 g, sodium 1.8 g, magnesium 0.52 g, vitamin A 21 017 IU, vitamin D_3 2312 IU, vitamin E 70.1 mg, vitamin K 5.7 mg, vitamin B_1 5.7 mg, vitamin B_2 9.2 mg, vitamin B_6 7.0 mg, vitamin B_{12} 70.0 µg, niacin 6.6 mg, pantothenic acid 18.9 mg, biotin 84.1 µg, cholin chloride 350.0 mg, zinc oxide 103.3 mg, copper 17.5 mg, iron 145.3 mg, manganese 96.3 mg, potassium iodate 2.1 mg, selenium 0.3 mg

starch. Two equal meals were fed to the animals daily at 8:00 and 20:00 h at a total level of 35 g/kg (as fed) of their individual BW. They were offered in mash form, and were mixed with water (1/1 w/v) prior to feeding. Water was available for *ad libitum* intake.

Experimental procedure

Ileal digesta was collected consecutively for a total of 24 h, from 8:00 on day 5 to 8:00 on day 6. The collection procedure was adapted from Li et al. (1993) using plastic tubing attached to the barrel of the cannula by elastic bands. The bags were changed at least every 20 min. Two ml of 2.5M formic acid were added to those sampling bags which were used for measurements of nutrient digestibilities to minimize further bacterial fermentation and immediately frozen at –30°C. Ileal digesta samples for the determination of nutrient digestibilities were pooled within each animal, freeze-dried, and ground to 0.5 mm. For the measurement of microbial metabolites, i.e. short chain fatty acids (SCFA), lactic acid, and ammonia, and pH, approximately 5 g of digesta were taken every 2 h (8:00, 10:00, 12:00, 14:00, 16:00, 18:00, 20:00, and 22:00 on day 5 and 00:00, 2:00, 4:00, 6:00 and 8:00 on day 6) and immediately frozen at –30°C. Digesta samples for the measurement of SCFA and lactic acid were pooled for each animal prior to analyses. For determination of bacterial numbers, digesta samples were collected between 5 and 6 h postprandially. After digesta had been emptied into plastic tubes, digesta samples were immediately kept on an ice-salt solution and transferred within 10 min to a freezer to be stored at –80°C until analysis.

Analytical procedures

Analyses of nutrients. Determination of dry matter (DM), ash, crude protein (CP), neutral detergent fibre (NDF), and acid detergent fibre (ADF) of the assay diets and ileal digesta samples was performed according to VDLUFA procedures (Naumann and Bassler, 1997). Concentrations of TiO_2 in the assay diets and in ileal digesta samples were analyzed according to the method described by Brandt and Allam (1987). The ileal digestibility of proximate nutrients was calculated using TiO_2 as an indicator according to the following equation:

$$D = 100\% - [(I_D \times A_F)/(A_D \times I_F) \times 100\%]$$

where:
D = nutrient digestibility (%)
I_D = marker concentration in the assay diet (g/kg DM)
A_F = nutrient concentration in ileal digesta (g/kg DM)
A_D = nutrient concentration in the assay diet (g/kg DM)
I_F = TiO_2 in ileal digesta (g/kg DM)

Analyses of microbial metabolites. The SCFA concentrations in ileal digesta were measured by gas chromatography (HP 6890 Plus GC-System) (Agilent Technologies, Santa Clara, USA) using 4-methyl-iso-valeric acid as internal standard. Digesta samples were prepared according to principles described by Zijlstra et al. (1977). The lactic acid concentrations (D-lactic acid, L-lactic acid) were determined by means of a photometric test kit (No. 1112821) (Boehringer, Ingelheim, Germany). Ammonia concentration in ileal digesta was measured with the aid of a gas-sensitive electrode, combined with a digital voltmeter (Mettler-Toledo, Giessen, Germany). The branched-chain proportion (BCP), which was used as indicator for the degree of protein fermentation (Macfarlane et al., 1992), was calculated as follows:

BCP (%) = (isobutyric + isovaleric)/total SCFA × 100%

DNA extraction and quantitative PCR. Extraction of total genomic DNA of ileal samples was performed by means of a Fast DNA® SPIN Kit for Soil (MP Biomedicals, Heidelberg, Germany) according to the manufacturer's instructions and as described in detail by Metzler-Zebeli et al. (2009).

Quantitative real-time PCR was performed using previously published primer sets and annealing temperatures for *Lactobacillus* spp. (forward: 5'- AGCAGTAGGGAATCTTCCA, Walter et al. (2001); reverse: 5'- CACCGCTACACATGGAG, Heilig et al. (2002)), and *Enterobacteriaceae* (forward: 5'- GTTAATACCTTTGCTCATTGA; reverse: 5'- ACCAGGGTATCTAATCCTGTT; probe: 5'- FAM-CGTGCCAGCAGCCGCGGTA-DABCYL, Malinen et al. (2003)). The primers were purchased from Eurofins MWG Operon (Straubing, Germany). The quantification of *Lactobacillus* spp. and enterobacteria was performed using an iCycler iQ™5 Realtime Detection System (Bio-Rad Laboratories GmbH, Munich, Germany), associated with the iCycler Optical System Interface software (Version 2.0, Bio-Rad Laboratories GmbH). The reaction mixture consisted of 12.5 µl iQ™SYBR® Green Supermix (Bio-Rad Laboratories GmbH), 1 µl of each primer (10µM), 10.5 µl milliQ water for SYBR® Green assays, and 1 µl of template DNA. For Taqman assays, the reaction mixture consisted of 12.5 µl iQ™ Supermix® (Bio-Rad Laboratories GmbH), 1 µl of each primer (10µM), 1 µl probe (10µM), 9.5 µl milliQ water, and 1 µl of template DNA. Standard curves were generated using serial dilutions of extracted DNA of bacterial cell cultures of known quantity as based on cell counts. The following strains were used to generate calibration standards: *Lactobacillus mucosae* DSM 13345, *Lactobacillus reuteri* DSM 20016, *Lactobacillus acidophilus* DSM 20079, *Lactobacillus amylovorus* DSM 20531, *Lactobacillus brevis* DSM 20054, and *Escherichia coli* DSM 613. For *Lactobacillus* spp. the standard was generated using a combination of the listed strains. Amplification conditions were 95°C for 5 min for initial denaturation, followed by 40 cycles of denaturation at 95°C for 15 s, primer annealing for 30 s (depending on optimal temperatures) and extension at 72°C for 30 s, and a stepwise increase of the temperature from 55 to 95°C to obtain melting curve data. Melting curves were checked after amplification to ensure single product amplification of consistent melting temperature. In addition to the correct melting profiles, PCR amplification products were verified by gel electrophoresis (2% agarose). Data collection was at the extension step. Quantification was performed in duplicate, and the mean values were calculated. Results were reported as $\log_{10}$ cells/g fresh matter.

Statistical analyses

The data were subjected to Analysis of Variance using the MIXED Procedure of SAS (Statistical Analysis System, Version 9.3, 2008). The model was as follows:

$$y_{ijk} = \mu + \beta_{ij} + e_{ijk}$$

where:
y_{ijk} = j^{th} measurement on k^{th} animal in i^{th} treatment
μ = overall mean (fixed)
β_{ij} = effect of i^{th} treatment (fixed)
e_{ijk} = error associated with y_{ijk} (random)

The significance level for all Wald-type *F*-tests was set at a = 0.05. Significant differences between treatments were represented by different superscript letters using the algorithm for letter-

Table 2. Ileal dry matter and nutrient digestibilities in piglets fed a control diet or a diet supplemented with probiotics and prebiotics, or probiotics and xylanase (%)*

	Control	Pro/Pre	Pro/Xyl	SEM	*P*-value
Dry matter	67.5	65.7	71.1	1.70	0.126
Ash	38.2	34.1	44.7	4.57	0.306
Crude protein	73.3	73.3	75.8	2.29	0.681
Neutral detergent fibre	30.5[a]	27.9[a]	43.2[b]	3.85	0.045
Acid detergent fibre	5.8	6.3	21.2	5.51	0.134

Pro/Pre = control plus probiotic and prebiotic, Pro/Xyl = control plus probiotic and xylanase
[a,b]within a row, Least Squares Means values without a common superscript differ ($P < 0.05$)
*data are expressed as Least Squares Means with pooled SEM; $n = 4$ per treatment

based representation of all pair-wise comparisons according to Piepho (2004).

RESULTS

The piglets readily consumed their feed allowances, and there were no signs of diarrhoea. The analyzed chemical composition of the basal diet amounted to 893 g/kg DM, and 198, 70, 164, and 51 g/kg DM for CP, ash, NDF, and ADF, respectively.

The effects of dietary supplementation with Pro/Pre and Pro/Xyl on ileal nutrient digestibility are shown in Table 2. The supplementation of Pro/Pre did not affect ileal DM and nutrient digestibilities ($P > 0.05$). However, supplementation of Pro/Xyl increased ileal digestibility of NDF ($P < 0.05$) by 41.6%, compared with the control treatment.

Ileal concentrations of the microbial metabolites (Table 3), including D-lactic acid, L-lactic acid, and total lactic acids, total and individual SCFA, the BCP, as well as ammonia concentration did not differ between dietary treatments ($P > 0.05$). The pH of ileal digesta (approximately 7.2) was also not affected ($P > 0.05$).

Bacterial numbers of *Lactobacillus* spp. and *Enterobacteriaceae* ($\log_{10}$ cells/g fresh matter) in ileal digesta, as well as the ratio of lactobacilli to *Enterobacteriaceae*, are shown in Table 4. *Lactobacillus* spp. populations were not affected ($P > 0.05$) by any of the dietary treatments. Also, there was no effect on bacterial cell numbers of *Enterobacteriaceae* upon dietary supplementation with Pro/Xyl ($P > 0.05$). However, these numbers were significantly reduced when Pro/Pre was added to the basal diet, resulting in an increased ileal lactobacilli to enterobacteria ratio ($P < 0.05$), compared with the control treatment.

DISCUSSION

Ileal nutrient digestibility

An improvement in ileal digestibility of NDF ($P < 0.05$), as observed in the present study due to supplementation with Pro/Xyl, is in accordance with previous studies in piglets, where dietary supplementation of xylanase increased ileal digestibility of NDF, but of crude fibre (CF) and CP as well (Diebold et al., 2004). Cereals such as wheat and barley, which are commonly used feedstuffs in pig diets, contain substantial amounts of NSP, for example arabinoxylans, that may act as anti-nutritional factors, thereby limiting nutrient di-

Table 3. Concentrations of microbial metabolites, branched-chain proportion (%), ammonia, and pH in ileal digesta of piglets fed a control diet or a diet supplemented with probiotics and prebiotics, or probiotics and xylanase*

Item	Control	Pro/Pre	Pro/Xyl	SEM	*P*-value
g/kg DM					
D-Lactic acid	12	12	9	3.7	0.747
L-Lactic acid	27	31	28	5.4	0.849
Total lactic acids	40	43	36	7.3	0.799
mmol/kg DM					
Acetic acid	144	100	167	20.8	0.123
Propionic acid	63	49	78	14.7	0.429
Isobutyric acid	0.6	0.4	1.3	0.3	0.116
Butyric acid	14	5	18	4.7	0.182
Isovaleric acid	0.6	0.4	1.5	0.4	0.177
Valeric acid	4	2	6	2.6	0.603
Total SCFA	226	157	271	40.8	0.195
BCP (%)	0.50	0.50	0.99	0.157	0.086
Ammonia (mmol/kg DM)	81	58	63	12.7	0.449
pH	7.2	7.3	7.1	0.1	0.490

Pro/Pre = control plus probiotic and prebiotic, Pro/Xyl = control plus probiotic and xylanase, DM = dry matter, SCFA = short-chain fatty acids, BCP = branched-chain proportion

*data are expressed as Least Squares Means with pooled SEM; $n = 4$ per treatment

Table 4. Cell numbers of *Lactobacillus* spp. and *Enterobacteriaceae* (log_{10} cells/g fresh matter), and ratio of lactobacilli to enterobacteria in ileal digesta of piglets fed a control diet or a diet supplemented with probiotics and prebiotics, or probiotics and xylanase*

	Control	Pro/Pre	Pro/Xyl	SEM	*P*-value
Lactobacillus spp.	9.1	9.8	9.2	0.44	0.433
Enterobacteriaceae	7.6[a]	6.0[b]	6.9[ab]	0.31	0.016
Lactobacilli : Enterobacteria	1.45[a]	3.86[b]	2.32[ab]	0.59	0.049

Pro/Pre = control plus probiotic and prebiotic, Pro/Xyl = control plus probiotic and xylanase

[a,b]within a row, Least Squares Means values without a common superscript differ ($P < 0.05$)

*data are expressed as Least Squares Means with pooled SEM; $n = 4$ per treatment

gestibility. Xylanase randomly cuts the arabinoxylan backbone into small fragments (Tapingkae et al., 2008; De Lange et al., 2010), thus improving digestibility and nutritional value of high-NSP diets (Diebold et al., 2004). For example, significant improvements in growth performance of piglets were reported when glucanase or xylanase were added to barley-based diets (Inborr et al., 1993; Li et al., 1996).

There is also some evidence that supplemental probiotics may increase nutrient digestibility in pigs. According to Giang et al. (2010a), supplementation of a lactic acid bacteria complex with probiotic properties to a maize-soybean meal-based diet for weaned piglets resulted in an increase of ileal apparent digestibility of CP, CF, and organic matter (OM) as well as of total tract apparent digestibility of CP and CF. Furthermore, dietary supplementation with a combination of a probiotic complex containing lactobacilli and yeast alone or fed together with an enzyme cocktail (amylase, protease, cellulase, β-glucanase, and xylanase) improved total tract digestibility of CP, CF, OM and growth performance in weaned pigs compared to the control (Giang et al., 2010b). In the present study, the combined supplementation of probiotic and prebiotic did not affect ileal nutrient digestibility including NDF digestibility. Thus, the observed increase in ileal NDF digestibility obtained for the Pro/Xyl treatment appears to be due to the action of xylanase rather than the supplemental probiotic. It has to be emphasized, however, that in the study of Giang et al. (2010b) a mixture of different probiotic strains and several enzymes was used to determine their impact on total tract digestibility, whereas in the present study a single probiotic strain and one particular enzyme were used to assess their effect on ileal rather than total tract nutrient digestibilities. Comparisons between the results of both studies are biased because multi-strain or multi-species probiotics are supposed to have more effective and consistent functionality than mono-strain or single-species probiotics, at least when applied in rodents, chickens, or humans (Timmerman et al., 2004). Studies in pigs comparing effects of different probiotic strains as well as multi-strain/multi-species preparations, in combination with NSP-hydrolyzing enzymes, are still lacking.

Bacterial numbers and microbial metabolites

In the present study, the combined supplementation of Pro/Pre did neither change lactobacilli numbers nor the concentration of microbial metabolites at the ileal level. In general, dietary supplementation of pre- and probiotics is assumed to stimulate saccharolytic activities of the intestinal microbiota and to promote growth of lactobacilli (De Lange et al., 2010), however certain restrictions may apply. For example, there is evidence that the efficacy of prebiotics depends on the dose used. According to a study of Metzler-Zebeli et al. (2009), no effects on bacterial populations in the ileum of weaned pigs could be observed, when adding the prebiotic inulin at 0.2% to a diet mainly consisting of wheat, barley, and soybean meal. However, as in the present study, a considerably higher dose of a prebiotic (oligofructose, 2%) was added to the diet, when compared to the 0.2% used by Metzler-Zebeli et al. (2009), the observed lack of response appears to be related to other factors. For example, basal diets consisting of fructan-rich ingredients such as e.g. wheat (Van Loo et al., 1995) have been suggested to lead to a masking effect when testing prebiotics such as oligofructose or inulin (Metzler-Zebeli et al., 2009). Furthermore, under optimized health and housing conditions (Mikkelsen et al., 2003), there is eventually only little response in view of high numbers of lactic acid bacteria already present

in the GIT (Franklin et al., 2002; Loh et al., 2006). Also, in the present study, additives were supplied for a rather short period (5 days) to assess their potential in the period immediately after weaning, similar to the use of in-feed antibiotics. It appears that at least some members of the microbial ecosystem (i.e. lactobacilli) would have needed more time for proliferation. Accordingly, Castillo et al. (2007) have suggested that the intestinal microbiota may need a prolonged adaptation period to adapt completely to dietary changes. On the other hand, in the present study numbers of *Enterobacteriaceae* were significantly reduced, despite the short-term application in the present study. As a result, an increased ileal lactobacilli to enterobacteria ratio was obtained compared with piglets fed the control diet. Lactobacilli are considered to be beneficial for maintaining intestinal health due to their ability to control potential pathogenic groups, such as *E. coli* (Canibe and Jensen, 2003), and to optimize immune response (Perdigon et al., 2001). Accordingly, the ratio of lactobacilli to enterobacteria may be used as an index of intestinal equilibrium (Hillman et al., 1995). Similar to the results of the present study, Le Bon et al. (2010) observed no effect on faecal lactobacilli levels, whereas *E. coli* and coliform counts were significantly reduced in piglets fed a successive probiotic supplement consisting of *Saccharomyces cerevisiae* ssp. *boulardii* and *P. acidilactici* for four weeks. Thus, results on *Enterobacteriaceae* numbers in the present study were obviously comparable to the study of Le Bon et al. (2010) using a much longer application period, suggesting that *P. acidilactici* can be considered as promising alternative to the use of in-feed antibiotics. *Pediococcus acidilactici* has been shown in weaning piglets to increase villi height and crypt depth in treated animals in comparison with controls, indicating a protection of the piglet's small intestinal mucosa, thereby improving local resistance to infections (Di Giancamillo et al., 2008). Furthermore, *P. acidilactici* was effective in reducing attachment of enterotoxigenic *E. coli* harbouring the F4 (K88) fimbriae (ETEC F4) to the ileal mucosa of weaned pigs, a key step in the development of disease (Daudelin et al., 2011). These results suggest possible mechanisms for its ability to modulate the intestinal microbiota.

Originally, it was hypothesized that xylanase may hydrolyze, at least in part, NSP, thereby producing oligosaccharides that might act as prebiotics and affect composition and metabolic activity of the intestinal microbiota (Vahjen et al., 1998; Pluske et al., 2002; Högberg and Lindberg, 2004). However, in the present study, dietary supplementation of Pro/Xyl had no effect on ileal bacterial numbers or metabolites. Similarly, Diebold et al. (2004) did not observe any effects of xylanase supplementation to piglets' diet on microbial metabolite concentrations (D- and L-lactic acid, SCFA, and ammonia) in ileal digesta of piglets. It has to be considered that in the present study, as well as in the study of Diebold et al. (2004), piglets were kept individually in metabolic crates under optimized hygienic conditions. Thus, under more practical conditions, including an increased challenge by pathogens, effects on microbial activity and composition might be expected.

CONCLUSION

According to the results of the present study, dietary supplementation of Pro/Xyl improved NDF digestibility. Furthermore, supplementation of Pro/Pre decreased ileal *Enterobacteriaceae* numbers, thereby increasing the ileal lactobacilli to enterobacteria ratio compared to piglets fed the control diet. Obviously, the synbiotic approach is capable of steering the microbial composition towards an improved intestinal equilibrium at the ileal level. However, both treatments, i.e. Pro/Xyl and Pro/Pre failed to affect microbial metabolite concentrations and did not influence lactobacilli numbers determined in ileal digesta of weaned piglets. For detailed information on efficiency of alternative feed ingredients and their combinations, future studies should particularly include different probiotic strains (multi-strain, multi-species). Studies on potential gastrointestinal effects of feed additives (or their combinations) should include measurements at the ileal level, particularly with respect to changes of the microbial ecosystem of the upper digestive tract.

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Corresponding Author

Dr. Eva Weiss, University of Hohenheim, Institute of Animal Nutrition, 70593 Stuttgart, Germany
Tel.: +49 711 459 239 64, e-mail: eva.weiss@uni-hohenheim.de