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Effects of dietary supplementation with *Bacillus subtilis* and yeast culture on growth performance, nutrient digestibility, serum indices and faeces microbiota of weaned piglets

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ABSTRACT. The aim of this study was to investigate the effects of *Bacillus subtilis* and yeast culture (YC) on growth performance, nutrient digestibility, serum biochemical and immunological parameters and microbial community of weaned pigs. One hundred and ninety-two weaned piglets (Large White × Landrace, 96 males and 96 females) with an average initial body weight of 8.22 ± 1.46 kg were randomly divided into 4 groups (8 piglets per pen, 6 pens per group). Piglets in the control group were fed a basal diet with 1 g/kg of bacitracin zinc and the others were fed basal diets supplemented with 1 g/kg of solid-state products containing 10^{10} CFU/g of *B. subtilis* or 3 g/kg of YC with live yeast (*Saccharomyces cerevisiae*) cells of 4.5×10^6 CFU/g or the combination of two above mentioned supplements. Pigs were fed the experimental diets for 41 days. There was no significant difference in body weight and feed intake of piglets between control and other experimental groups. In comparison to the control group, the ether extract digestibility was increased in all supplemented group, and the P digestibility was decreased only in *B. subtilis* group. The supplementation with *B. subtilis* and/or YC did not affect either serum biochemical or immunological parameters in comparison to the control group. Also, the similar effect on faecal microbiota composition was stated in the supplemented groups and control one. *B. subtilis* or YC could be used as a substitute for antibiotics and so be applied to the rearing of weaned piglets. However, the usage of their combination in the examined proportion does not enhance the effect.

Introduction

Piglets weaning from sows are subjected to such stressors as diarrhoea, low feed intake and body weight loss, which reduce their immune response and disturb the equilibrium of the intestinal microflora owing to their immature immune and digestive systems (Alexopoulos et al., 2004; Ren et al., 2014). The antibiotic supplementation into feed for a long period may solve post-weaning problems, prevent animals from infectious diseases and improve the growth performance, and so it has turned into a global practice (Barton, 2000). The benefits of antibiotics in terms of efficiency in the gain of body weight, decrease in mortality and morbidity and reduction in the occurrence of subclinical diseases were observed during all phases of pig growth (Chattopadhyay, 2014). However, the widespread use of antibiotics has led to the emergence of drug-resistant bacteria, which will have a major health hazard to human health (Rather et al., 2012). Therefore, many countries have begun to ban the use of antibiotics in animal feed only to promote animal growth (Millet and Maertens, 2011).

As the most preferred and effective alternative for antibiotics in animal feeding, probiotics have been proved to have the beneficial effects on growth performance, nutrient retention and mortality and so they are widely used in poultry and livestock industry (Wang and Gu, 2010). Probiotics are live cultures of beneficial bacteria or yeast species which could compete with harmful gut flora colonization, maintain the gut integrity and stimulate the immune system of the host to increase the resistance to infectious agent (Benyacoub et al., 2003). *Bacillus subtilis* has demonstrated probiotic characteristics of pathogens inhibition and spore's high resistance to harsh conditions in the digestive tract of the host (Hung et al., 2012). Li et al. (2016) proved that *B. subtilis* could effectively improve the growth performance and feed conversion ratio (FCR) of broilers *via* the beneficial modulation of caecal microbiota. Recent studies showed that direct feeding of *B. subtilis* to pigs improved growth performance, nutrient digestibility, immune response and caecal microbiota (Lee et al., 2014; Canning et al., 2017). However, the effect of probiotics on the digestibility of protein and amino acids, and performance was varied with the energy density of diets (Kaewtapee et al., 2017).

Yeast culture is a dried product containing yeast and various metabolites of yeast fermentation. Previous studies indicate that yeast culture is a good candidate to be an antibiotic alternative improving growth performance and modulating gut immune response in weaned piglets (Shen et al., 2009; Trckova et al., 2014). Dietary supplementation of yeast culture has been proved to improve milk production of cows and growth performance of weaning pigs (Hansen et al., 2017; Kiros et al., 2018). However, in some studies it was reported that the addition of yeast culture did not affect the average daily gain (ADG), average daily feed intake (ADFI) or FCR of piglets and modified yeast culture (yeast culture + cell wall product (CWP) containing mannan oligosaccharides) would not improve the performance or health of weanling pigs (PeetSchwering et al., 2007).

Since the results of studies on the supplementation of *B. subtilis* and yeast culture products in pig nutrition are inconsistent, and the number of studies on the combined effects of *B. subtilis* and yeast culture in pigs is limited, the subject of the present study seems to be fully justified. Thus, the purpose of this study was to investigate the effects of *B. subtilis*, yeast culture and their combination on growth efficiency, nutrient digestibility and serum indicators in weaned piglets, and on the composition and diversity of faecal microflora studied with the use of high-throughput next-generation sequencing.

Material and methods

The research was conducted at the Fang Shan pig breeding farm (Beijing, China; latitude 39.67′N, longitude 116.19′E). The Chinese Academy of Agricultural Sciences Animal Ethics Committee approved the experimental protocol (AEC-CAAS-2017-03), and humane animal care and handling procedures were followed throughout the experiment.

Animals, diets and management

One hundred and ninety-two weaned piglets (Large White × Landrace, 96 males and 96 females) with an average initial body weight of 8.22±1.46 kg were randomly divided into four groups: (1) CON: piglets fed basal diet and 1 g/kg of bacitracin zinc (Zhongnongxing Feed Sci. & Tech. Co., Ltd, Beijing, China); (2) BS: piglets fed basal diet and 1 g/kg of solid-state products containing 10^{10} CFU/g of *B. subtilis*; (3) YC: piglets fed basal diet and 3 g/kg of yeast culture with live yeast (*Saccharomyces cerevisiae*) cells of 4.5×10^{6} CFU/g; (4) BY: piglets fed basal diet supplemented with 1 g/kg of solid-state products containing 10^{10} CFU/g of *B. subtilis* and 3 g/kg of yeast culture. The *B. subtilis* and yeast culture were acquired from Huanong Biological engineering Co., Ltd. (Beijing, China). Each group had six replicates with eight piglets in each replicate. The basal diet was formulated according to the nutrient requirements of National Research

Table 1. Composition and nutrient level of the basal diet, dry matter basis

Indices	Content
Ingredients	
maize	60
soybean meal	25
wheat bran	3
concentrate supplement [a]	8
premix [b]	4
Nutrient content [c]	
DM	90.76
CP	18.46
ME, MJ/kg	16.57
EE	4.03
Ash	7.31
Calcium	1.07
Phosphorus	0.42

[a] the concentrate used in this research was the milk replacer involved in patent (not open to the public); [b] the premix provided the following nutrients per kg of the diet: IU: vit. A 5512, vit. D 640, vit. K_3 2.2, vit. E 20; mg: vit. B_1 1.5, vit. B_2 5.5, vit. B_6 2.2, D-pantothenic acid 14.8, nicotinic acid 30.3, biotin 0.05, choline 500, Cu 50, Fe 100, Mn 10, Zn 50, I 0.85, Se 0.25; µg: vit. B_{12} 27.6; [c] nutrient levels are all measured values except ME that was calculated; DM – dry matter; CP – crude protein; ME – metabolic energy; EE – ether extract

Council (NRC, 2012). Feed and water were available *ad libitum* during the 41-day feeding period. The ingredients and chemical composition of the diet are presented in Table 1. Feed intake and body weight were measured at the beginning and end of the experiment to calculate ADG, feed intake (FI) and FCR.

Faeces and feed samples

The nutrient digestion trial was conducted during the last 5 days of the study. The feed samples were collected before morning feeding. Approximately 200 g of faecal samples were collected from each pen into sterile plastic bottles. The faecal samples were collected twice daily for 5 following days. The 5-day samples were homogenized and 500 g faecal sample of the total was collected and stored at −20 °C for further analysis of nutrient content. Digestibility estimates were measured using the indicator (acid-insoluble ash, AIA) method according to the procedure of Favero et al. (2014).

Chemical analyses

Chemical analyses were performed as it was described before (Favero et al., 2014). The dry matter (DM) was determined by the method of AOAC (1990). The gross energy (GE) was determined by an automatic adiabatic oxygen bomb calorimeter (C200; IKA Works Inc., Staufen, Germany). The nitrogen (N) content was determined by the Kjeldahl method, with selenium (Se) as a catalyst, and crude protein (CP) content was calculated as 6.25 × N (method 984.13; AOAC, 1990). The contents of ash (method 942.05), ether extract (EE; method 920.39), phosphorus (P; method 965.17) and calcium (Ca; method 968.08) were conducted according to the methods of AOAC (1990).

Blood samples

Blood samples were collected before morning feeding at the end of trial by precava venipuncture into 10-ml vacuum tube (Tianming Medical Instrument Co., Ltd, Jiangxi, China). The samples were centrifuged (5000 *g*, 20 min, 4 °C) to obtain the serum which was frozen at −20 °C until further analysis. The serum biochemical parameters such as total protein (TP), alkaline phosphatase (ALP), blood urea N (BUN), glucose (GLU), cholesterol (CHO), immunoglobulin G (IgG), immunoglobulin A (IgA) and immunoglobulin M (IgM) were detected by an automatic biochemical analyser (Hitachi 7160; Hitachi Limited, Tokyo, Japan) at the Centre for Clinical Laboratory Development of the Chinese Academy of Medical Sciences (Beijing, China).

Microbial analysis

DNA extraction, PCR amplification of 16S rRNA and Hiseq sequencing. The faecal samples were collected from each pen *via* rectal massage, and microbial DNA was extracted using a commercially available kit (Omega Bio-tek, Norcross, GA, USA). The amplification and sequencing were conducted in Total Genomics Solution Technology Co., Ltd. (Shenzhen, China). The V3-V4 regions of the bacterial 16S ribosomal RNA genes were amplified using primers 341F (5'-barcode-CCTAYGGGRBGCASCAG)-3' and 806R (5'-GGACTACCVGGGTATCTAAT-3'), where barcode is an eight-base sequence unique to each sample. Amplicons were pooled in equimolar and paired-end sequenced (2 × 250) on an Illumina HiSeq platform after the purification using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) and quantification using QuantiFluor™ -ST (Promega, Madison, WT, USA).

Statistical and bioinformatics analysis

The effective tags were obtained after demultiplexed and quality filtered using the Quantitative Insights into Microbial Ecology (QIIME, v.1.8.0; Caporaso et al., 2010) and clustered into operational taxonomic units (OTU) by 97% similarity using UPARSE (version 7.1; Edgar, 2013). The α diversity and β diversity calculations were performed using QIIME. Taxonomic assignments of OTUs at 97% similarity level were conducted by QIIME against RDP (16S, http://rdp.cme.msu.edu/) database using confidence threshold of 80%. Variations in different groups were analysed using the SAS statistical software package (version 9.1, 2004; SAS Institute, Inc., Cary, NC, USA). The significance level was set at 0.05.

Results

Growth performance

There was no significant effect of *B. subtilis* or/and yeast culture supplementation on final body weight, ADG, FI and FCR in comparison to group fed diet with antibiotic ($P > 0.05$; Table 2). As compared to the CON group, the ADG of piglets in BS, YC and BY groups increased and were 14.14, 13.27 and 9.51%, respectively, but the differences were not significant.

Nutrient digestibility

The effects of supplementation of *B. subtilis* or/and yeast culture on digestibility of pigs are shown

Table 2. Effect of supplementation with *Bacillus subtilis* and yeast culture on growth performance of weaned piglets

Indices	Groups[1]				SEM	*P*-value
	CON	BS	YC	BY		
Initial body weight, kg	8.2	8.3	8.3	8.1	0.99	0.9993
Final body weight, kg	22.2	24.3	24.2	23.5	2.04	0.8807
Average daily gain, g	343	391	388	375	30.97	0.6841
Feed intake, g	795	864	873	843	63	0.8181
Feed conversion ratio[2]	2.32	2.23	2.25	2.24	0.08	0.8533

[1] groups: CK – control group, BS – *Bacillus subtilis* supplemented group, YC – yeast culture supplemented group, BY – both *Bacillus subtilis* and yeast culture supplemented group; [2] feed conversion ratio = feed intake (kg) / total body weight gain (kg)

in Table 3. The supplementation of *B. subtilis*, yeast culture or their combination increased the digestibility of EE, but the digestibility of P was decreased in BS group in comparison to CON group ($P < 0.05$). The digestibility of DM, organic matter (OM), ash and Ca tended to be lower in BS and YC groups than those in the CON group ($0.05 < P < 0.1$). No significant difference was observed in GE and CP digestibility among all groups ($P > 0.05$).

Blood metabolites and immune indices

There was no significant effect of *B. subtilis*, yeast culture or their combination supplementation on examined blood metabolites: TP, ALP, GLU, CHO and BUN, as well as on immune indices in comparison to control group fed diet with commercial antibiotic addition ($P > 0.05$; Table 4).

Microbial community

The species accumulation curves indicated that sampling had sufficient sequence coverage to accurately describe the bacterial composition of each group (Figure 1). The OTUs identified among the four groups is shown in Figure 2. Based on the OTUs at the 0.03 dissimilarity level, the indices of bacterial richness and diversity were determined by the method of Chao and Shannon (Dong et al., 2017). It was shown that addition of *B. subtilis* or/and yeast culture allowed to achieve the same bacterial richness and diversity in faeces of piglets as in the CON group ($P > 0.05$; Figure 2).

Based on the SILVA taxonomic database, the effective tags were classified from phylum to species. A total of 18 different phyla were detected

Table 3. Effect of supplementation with *Bacillus subtilis* and yeast culture on nutrient digestibility of weaned piglets

Indices	Groups[1]				SEM	*P*-value
	CON	BS	YC	BY		
GE, %	88.2	86.6	86.1	87.5	1.42	0.1377
DM, %	88.9	86.9	86.6	87.6	1.34	0.0577
OM, %	90.1	88.0	87.8	88.4	1.36	0.0580
CP, %	86.2	84.8	83.8	86.2	1.73	0.1098
EE, %	55.7^{b}	67.6^{a}	68.0^{a}	69.0^{a}	7.41	0.0138
Ash, %	72.8	67.7	68.6	71.8	3.34	0.0701
Ca, %	75.0	69.7	73.7	79.4	5.57	0.0866
P, %	64.0^{a}	54.4^{b}	57.0^{ab}	60.7^{ab}	5.24	0.0458

GE – gross energy; DM – dry matter; OM – organic matter; CP – crude protein, EE – ether extract; Ca – Calcium; P – Phosphorus; [1] groups: CON – control group, BS – *Bacillus subtilis* supplemented group, YC – yeast culture supplemented group, BY – both *Bacillus subtilis* and yeast culture supplemented group; [ab] – means within the same row with the different superscript letters are significantly different ($P < 0.05$)

Table 4. Effect of supplementation with *Bacillus subtilis* and yeast culture on serum biochemical indices of weaned piglets

Indices	Groups[1]				SEM	*P*-value
	CON	BS	YC	BY		
TP, g/l	65.2	65.9	66.2	70.9	8.60	0.7524
ALP, U/l	217	206	204	191	30.17	0.6203
GLU, mmol/l	2.80	3.03	3.68	3.84	0.86	0.1546
CHO, mmol/l	2.42	2.23	2.29	2.59	0.42	0.5809
BUN, mmol/l	7.56	7.29	7.28	7.60	1.01	0.9460
IgA, g/l	0.99	0.89	1.20	1.01	0.25	0.2670
IgG, g/l	8.56	8.37	8.49	8.98	0.75	0.6307
IgM, g/l	0.75	0.79	0.80	0.86	0.09	0.2146

TP – total protein, ALP – alkaline phosphatase, GLU – glucose, CHO – carbohydrate; BUN – blood urea nitrogen; IgA – immunoglobulin A; IgG – immunoglobulin G; IgM – immunoglobulin M; [1] groups: CON – control group, BS – *Bacillus subtilis* supplemented group, YC – yeast culture supplemented group, BY – both *Bacillus subtilis* and yeast culture supplemented group

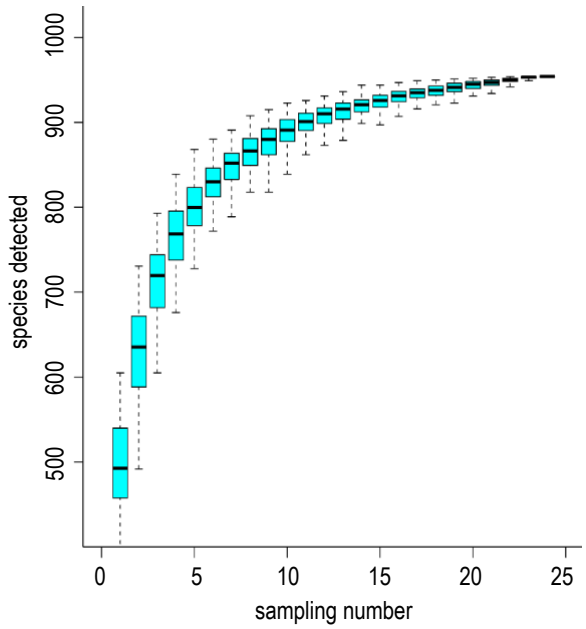

Figure 1. Species accumulation curves in faecal samples of piglets

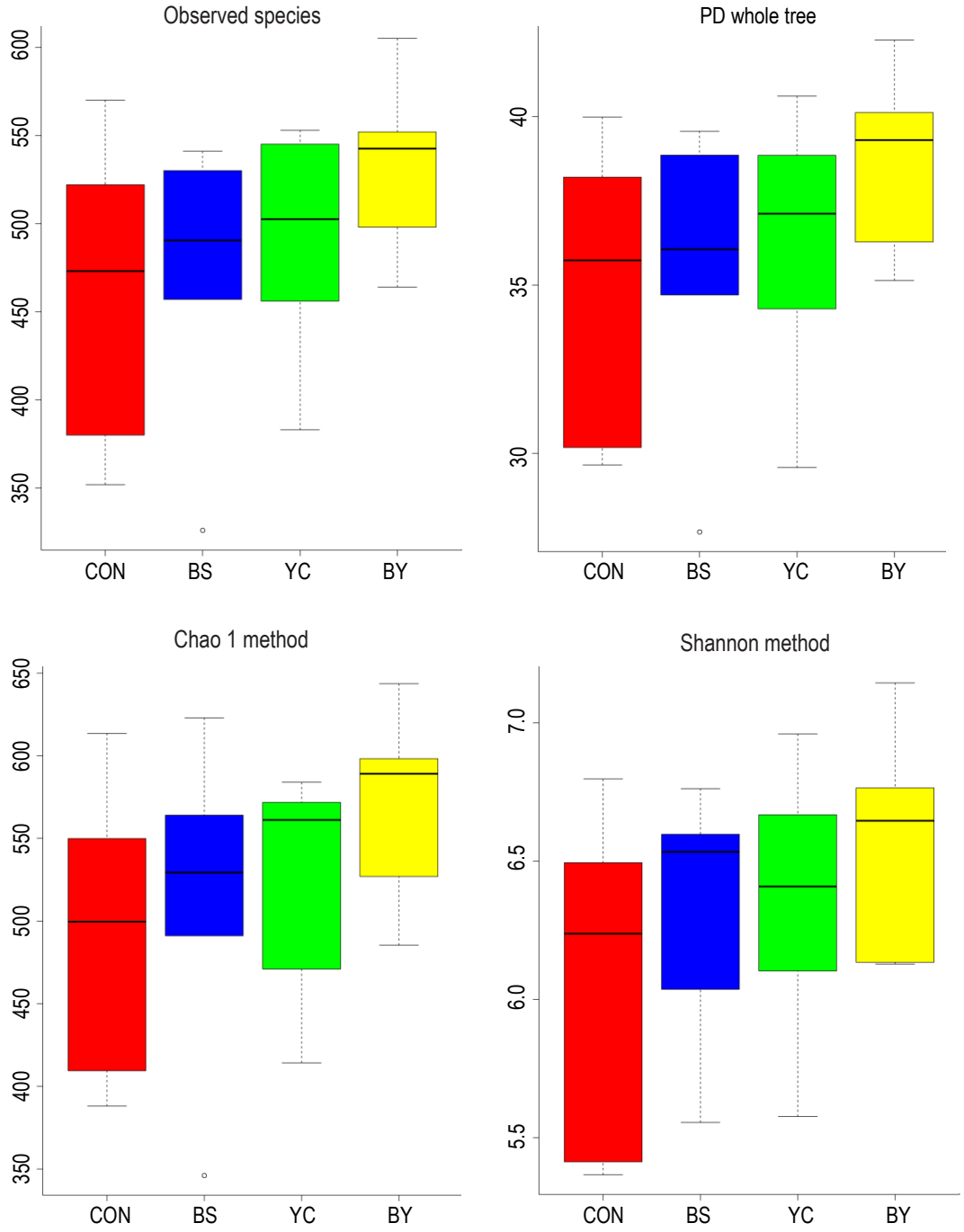

Figure 2. Community richness estimates and diversity indices for different treatments

in the examined samples (Figure 3; Table 5). The four groups showed very similar taxonomic compositions at the phylum-level distributions and the major sequences obtained from the samples belonged to *Firmicutes* and *Bacteroidetes*. In the control group with antibiotic addition, *Firmicutes*, *Bacteroidetes* and *Proteobacteria* were the dominant phyla and accounted for 51.3, 38.7 and 4.31 of the total reads, respectively. Within the BS group also *Firmicutes* (61.1%), *Bacteroidetes* (30.1%) and *Proteobacteria* (2.52%) were the dominant phyla. The YC group was dominated by *Firmicutes*, *Bacteroidetes* and *Actinobacteria*, representing 61.6, 28.0 and 2.98% of the total reads, respectively. However, in the BY group (like in the CON and BS groups) *Firmicutes, Bacteroidetes* and *Proteobacteria* were the most common groups and accounted for 58.0, 32.9 and 2.42% of the total reads, respectively.

A total of 88 different genera were detected in the examined faecal samples (Figure 4; Table 6). Analysis of the microbiota composition at genus level

Table 5. Effect of supplementation with *Bacillus subtilis* and yeast culture on the dominant phylum composition of weaned piglets, %

Indices	Groups[1]				SEM	*P*-value
	CON	BS	YC	BY		
Firmicutes	51.3	61.1	61.6	58.0	9.50	0.2145
Bacteroidetes	38.7	30.1	28.0	32.9	9.92	0.2837
Proteobacteria	4.31	2.52	2.83	2.43	2.42	0.5285
Euryarchaeota	2.67	2.18	2.86	2.11	2.41	0.9444
Actinobacteria	1.15	1.80	2.98	1.67	2.27	0.5812
Spirochaetes	1.11	1.22	0.79	2.11	1.67	0.585
Tenericutes	0.46	0.69	0.59	0.55	0.37	0.7784
Cyanobacteria	0.12	0.12	0.14	0.08	0.08	0.6242
WPS-2	0.044	0.020	0.004	0.015	0.05	0.6085
Fibrobacteres	0.057	0.033	0.022	0.029	0.04	0.5001
TM7	0.031	0.065	0.051	0.029	0.04	0.3149
Elusimicrobia	0.0065	0.0024	0.0119	0.0075	0.01	0.7516
Chlamydiae	0.0087	0.0022	0.0025	0.0018	0.01	0.2684
Deferribacteres	0.0028	0.0043	0.0009	0.0020	0.01	0.7982
Synergistetes	0.0041	0.0067	0.0023	0.0044	0.01	0.5441
Others	0.054	0.080	0.053	0.075	0.05	0.7509

[1] groups: CON – control group, BS – *Bacillus subtilis* supplemented group, YC – yeast culture supplemented group, BY – both *Bacillus subtilis* and yeast culture supplemented group

Table 6. Effect of supplementation with *Bacillus subtilis* and yeast culture on comparison of the dominant genus of weaned piglets, %

Indices	Groups[1]				SEM	*P*-value
	CON	BS	YC	BY		
Prevotellaceae	15.36	24.88	16.54	17.02	9.815	0.3336
Megasphaera	7.83	6.40	5.00	9.84	4.283	0.2458
Dialister	6.76	4.01	4.58	3.92	3.439	0.4689
Ruminococcus	0.87	1.00	4.09	2.14	3.104	0.2546
Lactobacillus	7.44	3.29	4.11	4.26	3.530	0.1842
Oscillospira	5.57	3.61	4.81	4.69	2.770	0.7072
Methanobrevibacter	2.82	2.63	2.07	2.12	2.379	0.9411
Streptococcus	1.18	1.42	2.50	2.60	1.786	0.4113
Treponema	0.78	1.11	2.11	1.21	1.669	0.5835
Roseburia	1.89	2.89	2.30	2.73	1.335	0.5924

[1] groups: CON – control group, BS – *Bacillus subtilis* supplemented group, YC – yeast culture supplemented group, BY – both *Bacillus subtilis* and yeast culture supplemented group

indicated that the most dominant bacteria in faecal microbiota in all four groups were *Prevotellaceae* and *Megasphaera*. However, the third dominant genus was *Lactobacillus* in CON group, *Dialister* in BS group and *Oscillospira* in both YC and BY groups. Besides the numerical differences between groups, the usage of *B. subtilis* and yeast culture caused statistically similar values for all detected genera.

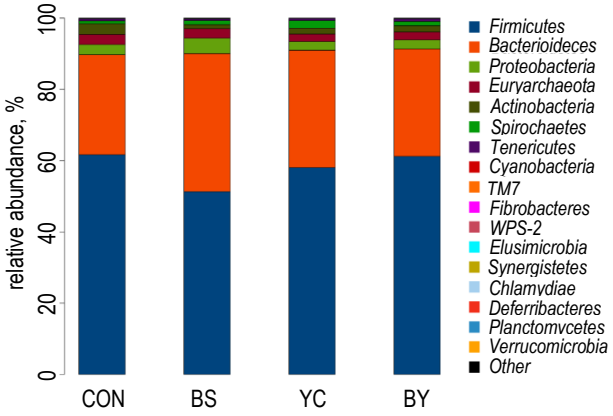

Figure 3. The bacterial composition at phylum level in faecal samples of piglets (colour-coded bar plots show the relative abundances of different phyla across different groups)

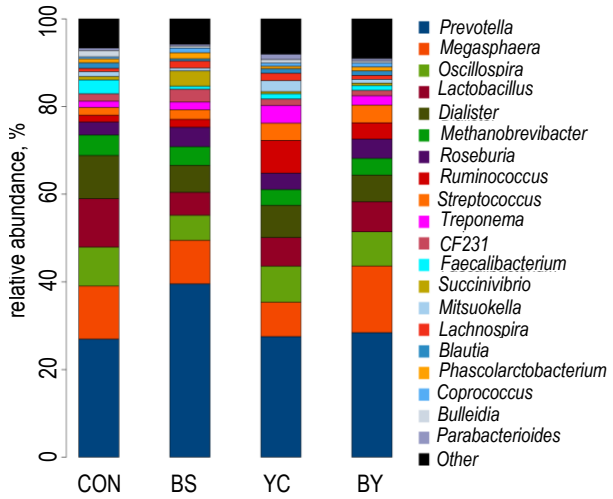

Figure 4. The bacterial composition at genus level in faecal samples of piglets (colour-coded bar plots show the relative abundances of different genera across different groups)

Discussion

Moore et al. (1946) were the first to discover that a low dose of antibiotics could promote the growth of chick, and the further studies with livestock showed the similar effects in swine and cattle. Addition of antibiotics to the feed was widely used in animal husbandry industry as an integral part of their feeding programmes. Spellberg et al. (2013) reported that 13 million kg of antibiotics were administered to animals and the majority of them were used as growth promoter of livestock. However, diseases that were thought to be fully controlled by antibiotics are now returning in new forms resistant to antibiotic treatment (Levy and Marshall, 2004). Evidences available in the literature speak volumes for the effective alternatives of probiotics for antibiotics in animal feeding.

As the members of probiotics, *B. subtilis* or yeast culture have the improving effect on the

growth performance of broilers (Hung et al., 2012), pigs (Jørgensen et al., 2016) and lambs (Tripathi et al., 2008; Estrada-Angulo et al., 2013). The results obtained in the present study indicated that the addition of *B. subtilis* or yeast culture as the substitution of antibiotic growth promoters allows to achieve ADG and FI comparable to the group with antibiotic supplementation. Korneagy et al. (1995) suggested that improvement in growth performance will be marginal during optimal rearing and feeding conditions and probiotics are more effective in animals during microflora development or when microflora stability is impaired. The efficacy of addition of materials might be influenced by the age and rearing regimes of pigs. In comparison to the antibiotic group, the addition of *B. subtilis* or/and yeast culture significantly increased the EE digestibility of weaned piglets. Jørgensen et al. (2016) indicated that the improvement in fat digestibility might be associated with the improved growth performance observed by dietary supplementation of *Bacillus*-based probiotic during the grower period. The serum biochemical indicators and immune parameters play an important role in the evaluation of the metabolism and immune status of animals. Previous studies reported that probiotics could stimulate the immune system by increasing the production of antibodies and activation of lymphocytes (Ng et al., 2009). Results from the present study showed that the concentration of IgA, IgG and IgM was no significant difference among the four groups. Kim et al. (2014) reported similar results that the addition of probiotics had no effect on serum immunoglobulins concentrations. The difference of bacterial strain, addition level and growth stage of pigs might be the causes leading to such distinction.

The mammalian intestine is colonized by trillions of microorganisms and most of which are bacteria that have co-evolved with the host in a symbiotic relationship (Kamada et al., 2013). The microbiota is a significant source of both nutritional metabolites and inflammatory innate immune signals (Gareau et al., 2010). The findings of the present study revealed that *Firmicutes*, *Bacteroidetes* and *Proteobacteria* are the dominant phyla in CON, BS and BY groups, which is in agreement with the previous studies in which *Bacteroidetes* and *Firmicutes* were numerically the most dominant phyla in the microbiome of terrestrial mammals (Levy and Marshall, 2004). Unlike in the groups with the addition of the examined supplements, *Lactobacillus* was the third dominant genus in the faecal of control group. This is in accordance with the previous studies stating that probiotics promoted the gastrointestinal colonization of beneficial bacteria and decreased the beneficial bacteria excreted in animal manure (Arena et al., 2014). The positive response to yeast in diet fed to swine may be a result of the ability of yeast to suppress the concentration of coliform bacteria in the intestinal tract of weanling piglets (White et al., 2002). Microorganisms such as enterotoxigenic *Escherichia coli*, *Salmonella*, *Streptococcus*, *Treponema* and *Roseburia* are agents potentially causing scours from weaning up to the end of fattening period. What's more, increased abundance of *Roseburia* is associated with weight loss and reduced glucose intolerance in mice (White et al., 2002). Results from the present study showed that addition of *B. subtilis* or/and yeast culture exhibits the same health-promoting effect as in group with antibiotic addition confirmed by the unchanged level of excreted *Streptococcus*, *Treponema* and *Roseburia* in faeces.

Conclusions

Dietary supplementation of *Bacillus subtilis* or yeast culture can improve the growth performance of the weaned piglets to the level similar to antibiotics, which can be connected with the increased digestibility of ether extract. *B. subtilis* or yeast culture can also ensure intestinal health by promoting the excretion of harmful bacteria with piglet faeces. It could be concluded that *B. subtilis* or yeast culture could be used as a substitute for antibiotics and so be applied to the rearing of weaned piglets. However, the usage of their combination in the examined proportion did not enhance the effect, so the coefficient optimum proportion needs to be optimized in further research.

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