Effect of a dietary probiotic blend on performance, blood characteristics, meat quality and faecal microbial shedding in growing-finishing pigs

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Abstract

The current study aimed to evaluate the effect of a dietary probiotic blend on growth performance, blood characteristics, meat quality, and faecal microbial population in growing-finishing pigs in a 12-week experiment. Pigs were assigned to two dietary treatments: a control basal-diet without probiotic blend (No-Pro) and a test-diet including the probiotic blend (Pro) according to pig bodyweight (BW) at the dose of 100 mg/kg of BW. Pigs fed the probiotics had higher final BW and increased average daily gain (ADG), as well as improved feed conversion ratio (FCR). Blood parameters of pigs were not affected by dietary probiotic complex. Meat crude protein and polyunsaturated fatty acids (PUFA) contents were increased in the group fed probiotics, whereas there were no significant differences in the other meat traits. Feeding of probiotics determined a decrease in faecal NH_3-N (+15.5%) and butyric acid concentrations, whereas no effects were observed on faecal acetic acid and propionic acid. An increased faecal *Lactobacillus* concentration was found when pigs fed probiotic blend. Based on our findings, feeding probiotic blend enhanced growth performance and meat quality in growing-finishing pigs, and also decreased faecal NH_3-N and butyric acid levels resulting in a viable approach to reduce animal excreta pollution.

Keywords: Diet, growth, pig, probiotics

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Introduction

Probiotics are defined as bio-preparations, including living metabolites or cells of stabilized autochthonous microorganisms, which optimize composition and colonization of gut microflora in animals and humans and have a stimulative effect on digestive processes (Fuller, 1989; Meng *et al*., 2010; Zhang *et al*., 2012). Because of the ban on the use of antibiotics, probiotics have been suggested as the most advantageous alternative for livestock because of their helpful effects. Probiotic bacteria *Lactobacillus* and *Bacillus subtilis* have been successfully applied to livestock species (Abdelqader *et al*., 2013). These two strains benefit animals by suppressing pathogens directly or indirectly (Bhardwaj *et al*., 2008; Seidavi *et al*., 2017). However, single species often expose limited functionality, leading to instability in aspects of the using effect (Zhang *et al*., 2017). A probiotic mixture consists of two or more live strains at different, and currently, probiotic mixtures have been reported to have better therapeutic effects than a single species (Wang *et al*., 2014; Zhang *et al*., 2017). Probiotics have been reported to improve growth and feed efficiency in piglets (Kyriakis *et al*., 1999; Yan & Kim, 2013) and in grower-finisher pigs (Meng *et al*., 2010). However, the use of probiotics to improve meat quality has been questioned, and results in pigs have been inconsistent (Quadros *et al*., 2001; Česlovas *et al*., 2005; Meng *et al*., 2010). Feed products that include probiotics can express beneficial effects such as the ability to inhibit the adhesion of the pathogens, mainly *E. coli* and other enteric pathogens (Coconnier *et al*., 1993; Zhang *et al*., 2012; Jahromi *et al*., 2016). In addition, the positive effect of probiotics on body cell immunity development in animals was documented (Zhang *et al*., 2003, Rossi *et al*., 2014; Wen *et al*., 2014; Mousavi *et al*., 2015). However, few studies have been conducted to evaluate the effect of dietary probiotic mixture on performance and meat quality of pigs.

Therefore, the objective of this study was to assess the effects of a probiotic blend on growth performance, blood parameters, meat quality and faecal microbial shedding of growing-finishing pigs.

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Materials and Methods

The probiotics preparation utilized in the present trial was obtained from a commercial company (SLAB51, Mendes SA, Lugano, Switzerland). The product is composed of a blend of these strains: *Streptococcus thermophilus* DSM 32245, mixture of two strains *Bifidobacterium animalis* ssp. *lactis* DSM 32246 and DSM 32247, *Lactobacillus acidophilus* DSM 32241, *Lactobacillus helveticus* DSM 32242, *Lactobacillus paracasei* DSM 32243, *Lactobacillus plantarum* DSM 32244, and *Lactobacillus brevis* DSM 27961.

The trial received ethical approval from the Italian Ministry of Health (n.597 /2015-PR del 23/06/2015) and was conducted in strict accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (Art. 18 D.L. 4 March 2014, no. 26).

A total of twenty mixed pigs [(Landrace × Yorkshire) × Talent] with average initial body weight (BW) of 22.80 ± 0.95 kg (SE) were used in a 12-week experiment. Animals were allocated to individual pens for performance evaluation. Pigs were assigned to two dietary treatments: the control basal diet without probiotic blend (No-Pro); and the experimental diet including the probiotic blend (Pro). The probiotics mixture was supplemented to pigs during the whole feeding period according to their BW at the dose of 100 mg/kg of BW. The basal diet was formulated to meet or exceed the nutrient requirements of pigs according to NRC (1998) as reported in Table 1.

Table 1 Chemical composition of the basal diet (as-fed basis) fed to growing-finishing pigs[1]

Metabolizable energy, kcal/kg	3,350
Crude protein, %	16.50
Crude fibre, %	4.60
Ether extract, %	5.00
Ash, %	5.80
Lysine, %	0.90
Methionine, %	0.45
Calcium, %	0.80
Total phosphorus, %	0.55
Sodium, %	0.25

[1]Ingredients: corn, barley, soybean meal (44% CP), wheat middlings, wheat bran, molasses, soybean oil, vitamin-mineral premix, monocalcium phosphate, calcium carbonate, sodium chloride.

Animals were housed in an environmentally controlled room with a concrete floor. All pens were equipped with a self-feeder and a nipple waterer to allow the pigs *ad libitum* access to feed and water throughout the feeding period. The BW of each pig was measured at the beginning of the experimental period and then every two weeks. Feed intake was recorded daily on a pen basis during the trial in order to calculate the average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR).

Blood samples were collected at the beginning of the trial from all subjects via jugular venipuncture and at the end of feeding experiment. Plasma samples, obtained after centrifugation at 4000 rpm × 20 min, were stored at -20 °C until the analysis. Blood samples were analysed with commercial kits (Sentinel Chemical, Milan, Italy) and an automatic spectrophotometer (Cobas FARA, Roche Diagnostic, Basel, Switzerland) for total protein, albumin, glucose, total cholesterol, high density lipoprotein (HDL)-cholesterol and low density lipoprotein (LDL)-cholesterol, urea, and triglycerides. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were assayed with commercial kits produced by Bio-Merieux (Marcy-l'Etoile, France) and Boehringer Mannheim (Maylan, France), following the methodology suggested by the producers. Red blood cell (RBC), total white blood cell (WBC), and lymphocytes were assayed with an automatic blood analyser (Advia-120, Bayer, NY, USA).

Faecal samples were collected at the end of the trial from the rectums of pigs and placed on ice for transportation to laboratory for immediate analysis. Faecal ammoniacal nitrogen (NH_3-N) concentration was determined according to the methods of Chaney & Marbach (1962). The volatile fatty acids (VFA) measured in this experiment included acetic acid, propionic acid and butyric acid, according to the method reported by Chen *et al*. (2005).

Faecal microbial shedding, including *Lactobacillus* spp. and *E. coli*, was determined on fresh faecal samples at the end of the feeding period. The samples were serially diluted tenfold in sterile saline (0.9%). Microbial assay of faecal samples was carried out by culture techniques. The microbial groups that were analysed were *Lactobacillus* spp. (de Man, Rogosa & Sharpe (MRS) agar), and *E. coli* (MacConkey (MAC) agar). The microbial plates were inoculated with three dilutions each in duplicate. The agar plates were incubated anaerobically at 37 °C for 24 hours, then microbial colonies were counted immediately. Microflora enumerations were expressed as log10 CFU/g.

The faecal consistency score was done on three randomly selected pigs in each dietary treatment using this 1–5-grade scoring system (Lan *et al*., 2016): 1 = hard, dry pellets in a small, hard mass; 2 = hard, formed stool that remains firm and soft; 3 = soft, formed and moist stool that retains its shape; 4 = soft, unformed stool that assumes the shape of the container; and 5 = watery, liquid stool that can be poured. Scores were recorded on a pen basis following observations of individual pig and signs of stool consistency in the pen. The score was reported as average daily faecal score of individual pigs.

At the end of the trial, pigs were slaughtered and the carcasses were chilled at 2 °C for 24 hours and a sample of the *Longissimus dorsi* muscle was removed. The meat samples were thawed at room temperature before evaluation. Samples were assayed for dry matter (method 945.15), ash (method 942.05), crude protein (N × 6.25, method 990.03), and crude fat (method 945.16) according to the procedures outlined by the AOAC (2000). Meat total lipids were extracted according to the chloroform–methanol (2:1 v/v) method of Folch *et al.* (1957).

In preparation for the analysis of fatty acid composition, meat samples (5 g each) were freeze-dried and then ground. Methyl heptadecanoate (Fluka, USA) was dissolved into n-hexane (1 mg/ml) as an internal standard. Methyl esters of the fatty acids were prepared by incubating samples (300 mg each) and 5 ml internal standard for two hours at 80 °C with methanolic acetyl chloride in a total volume of 9 ml (Sukhija & Palmquist, 1988). After cooling to room temperature, 7 ml of 7% (w/v) potassium carbonate was added with mixing, and then the organic phase was collected after centrifuging at 1500 *g* × 2 min at 4 °C. Fatty acid methyl esters were separated over a CP-SIL883 column (100 m × 0.25 mm i.d., film thickness 0.20 µm fused silica; Varian, Palo Alto, CA, USA) in a Shimadzu (Model 2GC17A, Kyoto, Japan) gas chromatograph with a HP GC Chem Station Rev. A.05.04 data handling system using flame ionization detection. Helium was used as the carrier gas at a constant flow rate of 1.7 ml/min. The oven temperature was programmed as follows: 175 °C, held for 4 min; 175–250 °C at 3 °C/min; and then maintained for 20 min at 250 °C. The injector port and detector temperatures were 250 °C. Samples (1 µl) were injected with an auto-sampler. Output signals were identified and quantified from the retention times and peak areas of known calibration standards. Values were expressed as percentages of the total fatty acids.

At 24 hours after euthanasia, the muscle pH was measured at a depth of 2.0 cm below the surface, using a combined glass-penetrating electrode (Ingold, Mettler Toledo, Greifensee, Switzerland). The 2-thiobarbituric acid-reactive substances (TBARS) were measured according to Witte *et al.* (1970), and the values were expressed as milligrams of malonaldehyde per kilogram of muscle.

Animals were considered the experimental unit and data were analysed using the one-way ANOVA procedure of SAS (2000). The means of the treatments were compared using *t*-test. Data variability was reported as pooled standard error of the means (SEM) and $P < 0.05$ was considered statistically significant.

Results and Discussion

Growth performance parameters of pigs such as final BW, average daily gain (ADG) and feed conversion ratio (FCR) were significantly improved after the administration of the probiotic blend SLAB51 (Table 2). Pigs fed the diet containing the probiotics had higher final BW compared with those in the control group ($P < 0.001$). The average daily feed intake (ADFI) was similar between groups during the total feeding period ($P > 0.05$). When ADG was calculated for the entire observation period, the pigs in the SLAB51-treated group performed significantly better than those of the control group ($P < 0.01$). When FCR was calculated for the entire trial period, the pigs fed SLAB51 had a better ($P < 0.001$) feed utilization than those in the control group.

Table 2 Effects of dietary supplementation with probiotics on growth performance in growing-finishing pigs

Item	No-Pro	Pro	Pooled SEM	*P*-value
Initial BW, kg	22.4	23.3	0.65	0.269
Final BW, kg	95.8	103.4	2.96	<0.001
ADG, g/d	815	890	11.6	<0.01
ADFI, g/d	1,980	2,040	31.5	0.058
FCR	2.43	2.29	0.21	<0.001

No-Pro: diet does not include probiotics; Pro: diet includes probiotics; BW: body weight; ADG: average daily gain; ADFI, average daily feed intake; FCR: feed conversion ratio.

Probiotic dietary supplementation was found to positively influence growth traits in growing-finishing pigs. However, the literature reported variable findings. Chen *et al.* (2005) suggested that average bodyweight gain was significantly improved by the addition of probiotics in growing pigs when fed 0.2% of probiotic blend in diet. According to the authors' research findings, Alexopoulos *et al.* (2004) and, more recently, Ross *et al.* (2010) and Yan & Kim (2013) found that dietary probiotics inclusion led to a higher ADG, ADFI, and FCR than in pigs fed no probiotics. As a result, the same authors suggested that the current probiotic blend has the potential to improve productive performance of growing-finishing pigs probably because of increased nutrient digestibility.

The influence of probiotic blend on blood characteristics in growing-finishing pigs is reported in Table 3. The evaluated haematology and serum chemistry parameters, including total protein, albumin, urea, glucose, total cholesterol, HDL- and LDL-cholesterol, AST and ALT, RBC, WBC and lymphocyte, were not affected by dietary treatments (P >0.05). Thus, dietary supplementation of probiotics indicated few or no significant evidence on pig blood characteristics. In agreement with the current findings, Chen *et al.* (2005), Meng *et al.* (2010), and Lan *et al.* (2016) stated that feeding a probiotic blend to pigs did not influence blood traits. Conversely, it was found that probiotics supplementation in pigs determined an improvement of red blood cells (Cho *et al*., 2005). Therefore, as stated by other authors, the various results related to the blood parameters are not yet understood. However, these conflicting results may be related to the sample size effect.

Table 3 Effects of dietary supplementation with probiotics on blood characteristics in growing-finishing pigs

Item	No-Pro	Pro	Pooled SEM	*P*-value
Total protein, g/dl	5.8	5.5	0.41	0.414
Albumin, g/dl	1.2	1.1	0.11	0.369
Urea, mg/dl	31.3	30.8	4.20	0.477
Glucose, mg/dl	60.9	61.8	7.27	0.229
Total cholesterol, mg/dl	74.6	73.1	9.51	0.545
HDL-cholesterol, mg/dl	38.5	37.0	5.17	0.442
LDL-cholesterol, mg/dl	39.5	39.0	5.93	0.254
Triglycerides, mg/dl	54.8	56.9	6.59	0.303
AST, IU/l	40.9	41.3	4.53	0.502
ALT, IU/l	48.5	47.9	4.42	0.287
RBC, 10^6/µl	7.2	7.3	0.44	0.651
WBC, 10^3/µl	13.9	14.2	0.68	0.396
Lymphocyte, %	55.5	56.3	4.01	0.597

No-Pro: diet does not include probiotics; Pro: diet includes probiotics; HDL: high-density lipoprotein; LDL: low-density lipoprotein; AST: aspartate aminotransferase; ALT: alanine aminotransferase; RBC: red blood cell; WBC: white blood cell.

The effects of probiotic blend on meat composition are reported in Table 4. The dry matter content of meat was not significantly different among treatment groups, and the crude protein content increased significantly in pigs fed probiotic blend (P <0.05). No significant differences were observed on meat crude fat and ash contents. Statistically significant differences between the feeding groups were found in the fatty acid profile in the lipid fraction of *longissimus dorsi* muscle. In fact, PUFA were increased (P <0.01) in the SLAB51-treated group. Thus, the intensified activity of lactic bacteria could positively influence the ingestion and absorption processes and indirectly influence the meat fatty acid profile. Meat pH and TBARS were unaffected by dietary treatment.

Table 4 Effects of dietary supplementation with probiotics on *Longissimus dorsi* meat muscle quality in growing-finishing pigs

Item	No-Pro	Pro	Pooled SEM	*P*-value
Dry matter, %	27.05	27.32	0.165	0.353
Crude protein, %	21.25	22.45	0.110	<0.05
Crude fat, %	2.17	2.03	0.095	0.265
Crude ash, %	1.21	1.15	0.011	0.412
Σ SFA, %	43.75	43.21	0.225	0.219
Σ MUFA, %	47.94	47.38	0.209	0.114
Σ PUFA, %	8.31	9.41	0.165	<0.01
pH	5.8	5.7	0.08	0.505
TBARS, mg of malonaldehyde/kg	0.07	0.06	0.001	0.753

No-Pro, diet not including probiotics; Pro: diet including probiotics: SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; TBARS: thiobarbituric acid reactive substances.

Some studies reported positive effects of probiotics on pig meat quality (Alexopoulos *et al*., 2004; Česlovas *et al*., 2005; Meng *et al*., 2010). On the contrary, others had negative findings (Quadros *et al*., 2001). These variable results of the effect of probiotics may be because of aspects such as bacteria strains, level of supplementation, composition of diet, feeding management, feed form and interaction with other dietary additives (Chesson, 1994; Chen *et al*., 2005; Meng *et al*., 2010). According to the current results, Česlovas *et al.* (2005) found that probiotics affected meat crude protein amount positively in pigs. Further, Rekiel *et al.* (2005) reported a positive influence of probiotic mixture in the fatty acid profile in the lipid fraction of *longissimus dorsi* muscle in fattening pigs, including the PUFA family. On the other hand, Batorska *et al.* (2003) did not find any effects of these additives on the meat fatty acid profile.

The effects of probiotic blend supplementation on faecal NH_3-N and volatile fatty acids (VFA) concentrations are reported in Table 5.

Table 5 Effects of dietary supplementation with probiotics on faecal ammoniacal nitrogen (NH_3-N) and volatile fatty acids concentrations of growing-finishing pigs

Item	No-Pro	Pro	Pooled SEM	*P*-value
NH_3-N, ppm	239.5	207.3	1.88	<0.01
Volatile fatty acids, % [1]				
Acetic acid	16.15	15.88	1.01	0.104
Propionic acid	12.85	13.21	2.12	0.125
Butyric acid	15.40	14.02	0.15	<0.05

[1] Percentage on total VFA; No-Pro: diet does not include probiotics; Pro: diet includes probiotics

Results showed that the NH_3-N level in the Sivoy®-treated group was decreased significantly (P <0.01). The acetic acid and propionic acid concentrations were not affected by the addition of probiotics in diet (P >0.05). However, butyric acid concentration was significantly reduced in pigs fed SLAB51 (P <0.05). Thus, based on these findings, dietary supplementation with probiotics proved to be a viable approach to decreasing animal excreta pollution, as stated by Chen *et al.* (2005). According to this study and the current trial, in a report by Jeon *et al.* (1996), it was found that feeding probiotic blend to pigs led to a significant reduction of faecal NH_3-N. In the present study, the authors detected a significant decrease in butyric acid, an important indicator of improved microbial activity.

As reported in Table 6, a significant increase (P <0.01) of faecal *Lactobacillus* concentration was observed with probiotics supplementation in pig diet, whereas no difference was detected regarding faecal *E. coli* concentration.

Table 6 Effects of dietary supplementation with probiotics on faecal microbial shedding and faecal score in growing-finishing pigs

Item	No-Pro	Pro	Pooled SEM	*P*-value
Lactobacillus, $\log_{10}$ CFU/g	7.09	7.18	0.069	<0.01
E. coli, $\log_{10}$ CFU/g	6.32	6.44	0.247	0.295
Faecal score[1]	3.31	3.26	0.138	0.366

No-Pro: diet does not include probiotics; Pro: diet includes probiotics.
[1] Faecal score 1–5, in which 1 = hard, dry pellets in a small, hard mass; 2 = hard, formed stool that remains firm and soft; 3 = soft, formed, and moist stool that retains its shape; 4 = soft, unformed stool that assumes the shape of the container; 5 = watery, liquid stool that can be poured (Lan *et al*., 2016)

Furthermore, the dietary treatments did not affect faecal score in pigs. In agreement with the current study, Yan and Kim (2013) and Lan *et al.* (2016) reported that faecal *Lactobacillus* increased by probiotics supplementation. However, Lan *et al.* (2016) observed a reduction in faecal *E. Coli* in weaning pigs fed probiotic blend. The findings of the current study confirm that the presence of *Lactobacillus* in the gastrointestinal tract is positively related to gut health and that the present probiotic blend (SLAB51) could be used as a good probiotic in growing-finishing pigs.

Conclusion

Based on the findings, the present study provides evidence for the positive influence of probiotics supplementation on growth performance and meat quality as well as faecal microbial shedding in growing-finishing pigs. An accurate assessment of the impact of probiotic blend in animal production and health should consist of an evaluation of the impact on other porcine productive parameters and health indexes. Thus, further definition of the interaction between the health status and dietary probiotics may aid in the design of the most effective probiotic blend for optimum animal production and health.

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Authors' Contributions

All the authors contributed from the onset of the study and approve of the final version.

Conflict of Interest Declaration

The authors have no conflict of interest to declare.

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