Effects of a mixture of *Bacillus amyloliquefaciens* and *Bacillus subtilis* on the performance of growing-finishing pigs

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Abstract

The study was conducted to determine the effects of a *Bacillus-based* probiotic (mixture of spores of *Bacillus amyloliquefaciens* (DSM 25840) and *Bacillus subtilis* (DSM 32324) supplementation on growth performance and health of growing-finishing (GF) pigs. A total of 576 GF pigs with initial body weight (BW) of 23.2 ± 2.95 kg were allotted to one of two treatments (control diet and probiotic diet). Pigs were blocked by litter origin, BW and sex and allotted to 24 mixed-sex pens (6 entire males and 6 females per pen) per treatment. The GF pigs were fed pelleted diets containing 0 (control diet) or 400 mg/kg (6×10^8 CFU per kg feed; confirmed by analysis) of the *Bacillus-based* probiotic. The diets were supplied ad libitum as dry feed. Pigs were followed till day 102 after the start of the study. During the grower phase (1-35 days), probiotic supplementation tended to improve the feed conversion ratio (FCR) ($P = 0.09$). During the finisher phase (35-102 days), probiotic supplementation significantly improved FCR ($P = 0.03$) and tended to increase the average daily gain (ADG) ($P = 0.09$). During the overall period (1-102 days), probiotic supplementation significantly improved FCR ($P = 0.01$). Probiotic supplementation did not affect the number of culled and veterinary treated pigs. The number of treatments due to ileitis (an infection with *Lawsonia intracellularis)*, however, tended to be lower in the probiotic group (7 vs 16; $P = 0.07$). Most pigs showed normal faecal consistency in the grower phase and the mean pen faecal score during the grower phase was similar in the control group and the probiotic group. In conclusion, feeding GF pigs diets supplemented with 400 mg/kg of a *Bacillus-based* probiotic containing a mixture of viable spores (confirmed by analysis before used in this trial) of two specific strains of *Bacillus amyloliquefaciens* and *Bacillus subtilis* improved the FCR of the GF pigs during the overall fattening period. Moreover, it tended to decrease the number of veterinary treatments due to ileitis.

Keywords: *Bacillus amyloliquefaciens*, *Bacillus subtilis*, growing-finishing pigs, performance

Abbreviations: AA, amino acids; ADG, average daily gain; ADFI, average daily feed intake; AID, apparent ileal digestibility; ATTD, apparent total tract digestibility; BW, body weight; DE, digestible energy; DM, dry matter; FCR, feed conversion ratio; GF, growing-finishing pigs; HBW, heavy body weight; LBW, light body weight; MBW, medium body weight; N, nitrogen

1. Introduction

Since the ban of antibiotics as growth promoters in the European Union in 2006, there has been an increased interest in using probiotics to support health and growth performance of pigs (Blavi et al., 2019). It has been extensively documented that probiotics can reduce digestive disorders and improve performance parameters (Ahasan et al., 2015; Bajagai et al., 2016). *Bacillus* spp. are commonly used as probiotics in animal feed (Larsen et al., 2014). Addition of *Bacillus*-based probiotics to the diet may improve feed efficiency and/or average daily gain (ADG) of growing-finishing (GF) pigs (Chen et al., 2005; Chen et al., 2006; Jørgensen et al., 2016; Bouwhuis et al., 2017). However, the effect of *Bacillus*-based probiotics on performance of pigs has been characterized as being inconsistent and with low reproducibility from farm to farm (Barba-Vidal et al., 2018). Larsen et al. (2014) characterized 245 bacterial isolates of *Bacillus* strains and concluded that isolates from *Bacillus amyloliquefaciens*, *Bacillus subtilis*, and *Bacillus mojavensis* showed the best overall characteristics in terms of heat resistance of spores, inhibitory activity against pathogenic bacteria and antibiotic resistance and, therefore, potential for usage as probiotic additives in feed. Blavi et al. (2019) tested the effect of *Bacillus amyloliquefaciens* (DSM 25840) and *Bacillus subtilis* (DSM 25841) on the digestibility of energy, protein and amino acids (AA) in growing pigs. Addition of *Bacillus amyloliquefaciens* to diets increased the apparent ileal digestibility (AID) of some AA compared with the control diet, whereas addition of *Bacillus subtilis* increased digestible energy (DE) of the diet. It can be suggested that supplementation of diets with a mix of *Bacillus amyloliquefaciens* and *Bacillus subtilis* may result in improved performance of GF pigs because of an improved utilization of both AA and energy. Jørgensen et al. (2016) investigated the effects of a mix of *Bacillus subtilis* and *Bacillus licheniformis* on the growth performance and apparent total tract digestibility (ATTD) of wean-to finish pigs. They concluded that supplementation of this mix improved ADG, feed conversion ratio (FCR) and ATTD of nutrients in pigs. Information about effects of a mix of *Bacillus amyloliquefaciens* and *Bacillus subtilis* on performance of GF pigs, however, is limited.

Therefore, the objective of this study was to study the effects of a *Bacillus*-based probiotic (mixture of viable spores of *Bacillus amyloliquefaciens* (DSM 25840) and *Bacillus subtilis* (DSM 32324)) on growth performance and health of GF pigs.

2. Materials and methods

The experiment was conducted at the Swine Innovation Center Sterksel (Sterksel, the Netherlands) from Wageningen Livestock Research (Wageningen, The Netherlands). The farm housing and husbandry were representative of EU farming conditions and met relevant ethical, hygienic and animal welfare requirements. Animals in this study were raised and treated according to Directive 2010/63/EU of 22 September 2010 (European Commission, 2010) and according to the recommendation of the European Commission 2007/526/CE (European Commission, 2007) covering the accommodation and care of animals used for experimental and other scientific purposes. The Institutional Animal Care and Use Committee of Wageningen Livestock Research approved the experimental protocol.

2.1. Animals, housing and experimental design

A total of 576 GF pigs (Large White boar x (York x Dutch Landrace) sow), average initial BW 23.2 $\pm$ 2.95 kg and average initial age 63 $\pm$ 0.6 days, were allotted to two treatments (Control diet and Probiotic diet) in two batches of 288 GF pigs each with three weeks in between. Pigs were balanced for litter origin, BW and sex and allocated to 24 mixed-sex pens (replicates) per treatment (12 mixed-sex pens/replicates per treatment per batch). Body weight was balanced by sorting pigs into blocks of light (L), medium (M) and heavy (H) BW, which were then allocated to L, M and H pens, then adjusted to equalize pen replicates for gender (6 males and 6 females per pen). Two pens in each block were then randomly allotted to the two experimental treatments. Pigs were housed in four fattening rooms. Each fattening room had 12 pens measuring 5 m x 2.5 m (1 m^2 per pig). Pen walls were partly open (at the back of the pens). To minimize cross-contamination, all six pens at one side from the aisle were allotted to the same treatment. The six pens at the other side from the aisle were allotted to the other treatment. Pigs were followed till day 102 after the start of the study. Environmental conditions, temperature and ventilation rate in the fattening rooms were automatically controlled and appropriate for the stage of the pigs.

2.2. Diets and feeding

Pigs were fed diets containing 0 (control group) or 400 mg/kg (6 x 10^8 CFU per kg feed) of the *Bacillus*-based probiotic. The probiotic was a mixture of viable spores of *Bacillus amyloliquefaciens* (DSM 25840) and *Bacillus subtilis* (DSM 32324) at a minimum concentration of 1.5 x 10^9 CFU/g and was produced by Chr. Hansen A/S (Hørsholm, Denmark). As the *Bacillus*-based probiotic was supplied in a $CaCO_3$ base, 400 mg/kg $CaCO_3$ in the control diets was exchanged with 400 mg/kg of the *Bacillus*-based probiotic. Before and in-between production of the diets a cleaning diet (cereal) was passed through the production line to minimize cross-contamination. To ensure homogeneous diets, 800 gram of the *Bacillus*-based probiotic was mixed with 5 kg of the basal diet before mixing in 2,000 kg batches of the diet. The GF pigs were fed a grower diet during the first five weeks and then a finisher diet till the end of the trial. The composition of the control diets is presented in Table 1. All diets were formulated with a low level of copper and no added organic acids, polysaccharidases, yeasts or probiotics other than the probiotic to be tested in the trial. All nutrients were supplied at normal concentrations, not exceeding EU maximum permitted concentrations for trace minerals or vitamins. The diets met Centraal Veevoederbureau (2012) nutrient recommendations for GF pigs. Pigs had ad libitum access to the pelleted diets and to drinking water. The diets were fed in a dry feed hopper with two feeding places.

2.3. Measurements

Pigs were weighed individually at the start of the trial and at days 35 and 102 (end of the trial). Total feed intake per pen was measured at the end of each feeding phase. Average daily gain, average daily feed intake (ADFI) and FCR were calculated from the start till the end of the trial and in both feeding phases. The number of pigs treated with antibiotics (Engemycine® 10% (4 ml per 50 kg BW), MSD Animal Health, Boxmeer, The Netherlands; Penject 30 (1 ml per 10 kg BW), Dopharma, Raamsdonkveer, The Netherlands) and the number of culled pigs were recorded. In general, pigs were treated for 3-5 consecutive days per treatment. Lame pigs were treated with Penject 30. The animal caretakers had years of experience with diagnosing ileitis caused by *Lawsonia* i*ntracellularis* (grey/black diarrhea and failure to grow) and treatments were based on their experience. Pigs with ileitis were treated with Engemycine® 10%. Faecal scores were performed weekly during the grower phase. In each pen the number of pigs with normal faeces (score = 0), pasty faeces (score = 1) and watery faeces (score = 2) was scored visually by the same person across the treatment groups (Van Nieuwamerongen et al., 2017). The mean score was calculated per pen per week. Thereafter, the mean score per pen during the five weeks was calculated. Diets were analysed for moisture by drying at 103 °C (European Commission, 2009), crude protein by using the Kjeldahl method (European Commission, 2009), ash by combustion to a constant weight at 550 °C (European Commission, 2009), crude fat after hydrolysis (European Commission, 2009), Cu (only the starter diet; NEN-EN 15510, 2017) and the number of CFU/kg diet of the added *Bacillus*-based probiotic (NEN-EN-15784, 2009).

2.4. Statistical Analysis

Performance parameters (BW, ADG, ADFI, FCR) and mean faecal scores per week (weeks 1 to 5) were analysed with pen as experimental unit using a two-way ANOVA procedure (GenStat, 2018). The model used was:

$$Y = \mu + \text{batch} + \text{block within batch} + \text{diet} + \varepsilon$$

where:

Y = dependent variable, μ = population mean, batch = batch effect (1, 2), block = block effect (1 to 24), diet = effect of dietary treatment (1, 2) and ε = residual error.

Data are presented as least square means. The number of culled pigs and pigs treated with antibiotics were analysed using the Chi-square test of SAS 9.3 (2011). Probability values of $P \leq 0.05$ were considered significant, whereas $0.05 < P \leq 0.10$ was considered as a tendency.

3. Results

3.1 Dietary ingredients

The levels of crude protein, crude fat, ash and Cu in the grower and finisher diet were as expected (Table 1). The CFU analysis confirmed the target CFU per kg of diet (less than 1.0 x 10^8 CFU/kg diet in the control diets and 4.37 x 10^8 and 5.25 x 10^8 CFU/kg diet in the grower and finisher diets with *Bacillus*-based probiotics, respectively).

3.2. Growth performance

In the probiotic group, one pen was deleted from the results because this pen was an outlier (the daily gain of the pigs in this pen was more than two time the standard deviation lower than the mean daily gain of the pigs in the probiotic group). During the grower phase (1-35 days), probiotic supplementation tended to improve FCR (P = 0.09), but it did not affect ADG and ADFI (Table 2). During the finisher phase (36-102 days), probiotic supplementation significantly improved FCR (P = 0.03) and tended to increase ADG (P = 0.09). During the overall period (1-102 days), probiotic supplementation significantly improved FCR (P = 0.01), but it did not affect ADG and ADFI.

3.3. Health and faecal scores

Probiotic supplementation did not affect the number of culled and individually veterinary treated pigs (Table 3). Pigs were only individually veterinary treated and not on pen level. The number of treatments with antibiotics did not differ between the control and the probiotic group (P = 0.12). The number of treatments due to ileitis caused by *Lawsonia intracellularis*, however, tended to be lower in the probiotic group (7 vs 16; P = 0.07). The mean number of treatment days (as percentage of total number of trial days) was not affected by probiotic supplementation. Mean pen faecal score during the grower phase was within normal range and similar in the control group and the probiotic group (Table 3).

4. Discussion

The *Bacillus*-based probiotic (mix of *Bacillus amyloliquefaciens and Bacillus subtilis*) improved FCR during both the grower and finisher period and the overall fattening period and tended to increase ADG during the finisher phase. In weaned piglets, a mix of *Bacillus amyloliquefaciens* and *Bacillus subtilis* also improved FCR and ADG (Cai et al., 2015) or only FCR (Jaworski et al., 2017). It hasn't been possible, however, to locate any published studies in which the effect of a mix of *Bacillus amyloliquefaciens* and *Bacillus subtilis* on the performance of GF pigs was studied. There are a few studies in which the separate effect of *Bacillus amyloliquefaciens* was tested in GF pigs. Bouwhuis et al. (2017) reported an improved FCR during the overall fattening period in GF pigs that were fed a diet with *Bacillus amyloliquefaciens* (DSM 25840). A positive effect of *Bacillus amyloliquefaciens* on nutrient digestibility might explain the improved FCR in our study and in the study of Bouwhuis et al. (2017). *Bacillus amyloliquefaciens* produce α-amylase (Gangadharan et al., 2008), cellulase (Lee et al., 2008) and proteases (Gould et al., 1975), which can improve the digestion of nutrients. In both growing and finishing pigs, Blavi et al. (2019) showed a greater AID of total indispensable, total dispensable and total AA in the diet supplemented with *Bacillus amyloliquefaciens* (DSM 25840) compared to the control diet. The improved AID of AA in both growing and finishing pigs might explain the improved FCR during the overall fattening period.

Improvement in FCR in GF pigs as a result of *Bacillus* supplementation may also be due to the impact of *Bacillus* on pig health through beneficial immune modulation (Davis et al., 2008), competitive exclusion of gastrointestinal pathogens, and secretion of the antimicrobial compounds that suppress the growth of harmful bacteria (Ji et al., 2013; Li et al., 2015). In our study, the percentage of pigs treated with antibiotics (11.5 vs 8.7% in the control and probiotic group, respectively; *P* = 0.28) did not differ significantly between the control and probiotic group. The number of treatments due to ileitis during the finisher phase, however, tended to be lower in the probiotic group (7 vs 16; *P* = 0.07). These results correspond with the results of Opriessnig et al. (2019), who showed that *Bacillus pumilus* and to a lesser degree *Bacillus amyloliquefaciens* and *Bacillus licheniformis* suppress a *Lawsonia intracellularis* infection. Thus, a better health might also contribute to the improvement in FCR.

In several studies (Alexopoulos et al., 2004; Ji et al. 2013; Zentek et al., 2017), the number of pigs with diarrhea was reduced when fed diets containing *Bacillus amyloliquefaciens* or *Bacillus subtilis*. Kim et al. (2019), however, showed that supplementation of *Bacillus subtilis* did not reduce the frequency of diarrhea. In our study most of the pigs had normal faecal consistency during the grower phase and the mean faecal score during the grower phase was not affected by probiotic supplementation. As mentioned earlier, during the finisher phase, the number of pigs treated due to ileitis (pigs with diarrhea and grey-dark faeces combined with growth reduction) was reduced by probiotic supplementation.

The effect of *Bacillus subtilis* was often studied in combination with other *Bacillus spp.* Supplementation of *Bacillus*-based probiotics including *Bacillus subtilis* resulted in an improved FCR in the grower phase and an improved ADFI and ADG during the finisher phase and the overall fattening period (Bouwhuis et al., 2017), an improved ADG and FCR during the grower phase, an impaired FCR during the finisher phase and an improved FCR during the overall fattening period (Jørgensen et al., 2016), an improved FCR during the finisher phase and the overall fattening period (Davis et al., 2008), an improved ADG and FCR during the grower phase and the overall fattening period (Alexopoulos et al., 2004), an improved ADFI and ADG during the grower phase (Wang et al., 2009) and an improved ADG during the grower phase

(Chen et al., 2005) and finisher phase (Chen et al., 2006). In most of these studies a positive effect of *Bacillu*s-based probiotics including *Bacillus subtilis* on the performance of GF pigs was found. However, in some studies no effect on the performance of GF pigs was shown. Moreover, in some studies *Bacillus*-based probiotics improved fat digestibility (Jørgensen et al., 2016), nitrogen (N) digestibility (Chen et al., 2015) or DE of the diet (Blavi et al., 2019) in growing pigs, whereas in other studies it decreased N digestibility (Blavi et al., 2019) in growing pigs or did not affect N digestibility in finishing pigs (Chen et al., 2006). The different effects of *Bacillus* based probiotics including *Bacillus subtilis* on performance and nutrient digestibility in GF pigs may be due to several factors, like differences in diet compositions, *Bacillus* strains, dose levels, age of the animals, sanitary status, genetics and interaction with environmental factors (Jørgensen et al., 2016; Barba-Vidal et al., 2018; Mingmongkolchai and Panbangred, 2018).

Overall, our results indicate that supplementation of diets fed to GF pigs with a mix of two specific strains of *Bacillus amyloliquefaciens* (DSM 25840) and *Bacillus subtilis* (DSM 32324) improve the FCR and may reduce the number of veterinary treatments due to ileitis.

5. Conclusions

Feeding GF pigs diets supplemented with 400 mg/kg (6 x 10^8 CFU per kg feed) of a *Bacillus*-based probiotic containing a mixture of viable spores of *Bacillus amyloliquefaciens* (DSM 25840) and *Bacillus subtilis* (DSM 32324) improved the feed conversion ratio of the pigs during the finisher period and the overall fattening period and tended to improve the feed conversion ratio during the grower period. Moreover, it tended to decrease the number of veterinary treatments due to ileitis (an infection with *Lawsonia intracellularis)*.

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Table 1

Ingredient and composition of the control diets (as-fed basis)[a]

	Grower diet	Finisher diet
Ingredient, g/kg		
Barley	252.60	252.60
Rye	50.00	100.00
Wheat	354.63	297.24
Maize	81.10	50.70
Wheat middlings	-	50.60
Rapeseed meal	49.90	79.80
Soybean meal	120.00	62.20
Sunflower seed meal	28.59	50.06
Palm oil	13.20	17.30
Soy oil	10.00	13.40
Limestone	10.10	7.49
Monocalcium phosphate	3.39	0.50
Sodium bicarbonate	2.20	-
Salt	4.52	4.09
DL-Methionine	1.25	0.30
L-Tryptophan	0.34	-
L-Lysine HCl	5.22	3.28
L-Threonine	1.99	0.87
Vitamin and mineral premix	6.00	5.60
Phytase	4.97	3.98
Analysed composition		
Dry matter, g/kg	903	901
Crude protein, g/kg	182	167
Crude fat, g/kg	43	47
Ash, g/kg	46	40
Cu, mg/kg	22	-
Calculated analysis		
Starch, g/kg	439.2	422.2
Metabolisable Energy, MJ/kg	14.33	14.33
Net Energy, MJ/kg	10.03	10.03
AID lysine[b], g/kg	10.1	7.8
Ca, g/kg	6.0	4.7
Cu, mg/kg	20.6	21.0
Mn, mg/kg	50.2	56.7
Zn, mg/kg	91.6	95.7
Vitamin A, IU/kg	6,533	6,533
Vitamin D3, IU/kg	1,608	1,608
Vitamin E, mg/kg	80.4	74.4
Se, mg/kg	0.2	0.2
Choline, mg/kg	22.6	45.6

Vitamin B2, mg/kg	4.0	4.0
Vitamin B5, mg/kg	9.0	9.0
Niacine, mg/kg	30.2	30.2
Vitamin B12, mg/kg	0.02	0.02

[a] Diets with *Bacillus*-based probiotic: 400 mg/kg limestone ($CaCO_3$) in the control diets was exchanged with 400 mg/kg of the *Bacillus*-based probiotic.

[b] AID lysine, apparent ileal digestible lysine.

Table 2
Growth performance[1] of growing-finishing pigs (average initial age 63 ± 0.6 days) fed a control diet or a diet containing 400 mg/kg of a *Bacillus*-based probiotic (a mixture of *Bacillus amyloliquefaciens* and *Bacillus subtilis*)

	Control diet	Probiotic diet	SEM	*P*-value
Body weight, kg				
At start	23.2	23.2	0.01	0.50
Day 35	51.1	51.2	0.22	0.79
Day 102	113.4	114.6	0.60	0.16
Grower phase (1-35 days)				
ADG[2], g	798	800	6.2	0.82
ADFI[2], kg	1.41	1.40	0.011	0.33
FCR[2]	1.77	1.75	0.009	0.09
Finisher phase (36-102 days)				
ADG, g	930	948	6.9	0.09
ADFI, kg	2.26	2.26	0.016	0.92
FCR	2.43	2.39	0.014	0.03
Overall (1-102 days)				
ADG, g	885	897	5.8	0.15
ADFI, kg	1.97	1.96	0.013	0.73
FCR	2.22	2.19	0.010	0.01

[1] Data represents LSmeans based on 24 replicates (pen is the experimental unit) in the control group and 23 replicates (pen is the experimental unit) in the probiotic group. In the probiotic group, one pen was regarded as an outlier.
[2] ADG, average daily gain; ADFI, average daily feed intake; FCR, feed conversion ratio.

Table 3

Health and faecal scores of growing-finishing pigs fed a control diet or a diet containing 400 mg/kg of a *Bacillus*-based probiotic (a mixture of *Bacillus amyloliquefaciens* and *Bacillus subtilis*)

	Control diet	Probiotic diet	SEM	*P*-value
No of pig at start	288	276		
Culled pigs, %	3.1	2.5	-	0.67
Number of pigs treated with antibiotics	33	24	-	0.28
Number of treatments with antibiotics[a]	38	25	-	0.12
Reason of treatment:				
Ileitis[b]	16	7	-	0.07
Lameness	17	15	-	0.81
Other reasons	5	3	-	0.60
Antibiotic treatment days[c], % of total number of trial days	0.36	0.30	-	0.18
Pen faecal score[d]	0.020	0.018	0.0036	0.71

[a] Three pigs in the control group were treated two times and one pig was treated three times during the experimental period of 102 days. In the probiotic group one pig was treated two times.

[b] All treatments due to ileitis were executed during the finisher phase.

[c] In general, pigs were treated for 3-5 consecutive days per treatment.

[d] Recorded weekly during the starter phase (5 weeks): normal faeces (score = 0), pasty faeces (score = 1), watery faeces (score = 2).