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Research Article
Effects of Dietary *Bacillus subtilis* Supplementation as Probiotics on Growth Performance and Nutrients Digestibility in Fattening Pigs

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Abstract
Objective: This research examined the effectiveness of two brands of probiotics in pig fattening diets, over three periods (starter, grower and finisher) on growth performance and nutrients digestibility. **Materials and Methods:** A total of 500 crossbred pigs [(Yorkshire-Landrace)×Duroc], with an average initial body weight (BW) of 22.70±1.2 kg at 56-58 days, were divided into five dietary treatments, according to a completely randomized design. The dietary treatments included, T1: basal diet (control group), T2: basal diet+*Bacillus subtilis* (*B. subtilis*) (1.0×10^{12} CFU kg^{-1}) at level 0.1% (Pro A) and basal diet+*B. subtilis* (7.0×10^{9} CFU kg^{-1}) at levels 0.0125%, 0.0250 and 0.0375% (Sanizyme®) were represented in T3, T4 and T5, respectively. **Results:** The results from these experiments show that the addition of two brands of probiotics improved the digestion and utilization of carbohydrates, proteins, fat, fiber and energy in feed components in the starter and grower periods of pigs ($p<0.05$). In the overall experimental time frame, improvements were found in the growth performance of average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR) in pig fattening diets supplemented with probiotics however, no significant differences ($p>0.05$) were observed when compared with the control group. **Conclusion:** The addition of *B. subtilis* creates a favorable response on growth performance and feed utilization and presented the highest economic return when compared with the control group.

Key words: Probiotics, pig fattening diets, feed efficiency, nutrients digestibility, economic benefits

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Health and feed are major factors driving profits in pig production. Under industrial feeding conditions, pigs are subjected to many stress factors, including transportation, vaccination, heat stress, etc. These conditions can lead to decreased growth performance, gut health and mortality[1,2]. Antibiotics have been widely used to prevent poultry and swine pathogenic bacterial diseases as well as improve meat and egg production. However, the use of antibiotics as a growth promoter (AGP) is banned in several countries, because the use of antibiotics in sub therapeutics may lead to the development of drug-resistant pathogens and drug-residues in animal products[3] and present an imbalance of normal microorganisms[4]. Continuous efforts are therefore being made to identify alternative strategies. Probiotics, prebiotics, synbiotics, organic acids and phytogenic feed additives could be such alternatives, with probiotics being one of the most suitable[5-9].

Probiotics are defined as "live microbial that when ingested in sufficient amounts confer an essential health helpful on the host[10]". During the last two decades, a renewed interest has been developed, concentrating on the role and effects of probiotics on animal production. It has been reported by many researchers that probiotics not only act as growth promoters, bioregulators and feed savers but also as immunostimulants useful to the animal's growth performance and health. The effects have been reported in pigs as well as in poultry, calves, rabbits and aquaculture[11-18]. Continuous usage of probiotics may support the non-specific immune system of animals and therefore, anti-infectious treatments should be reduced. Probiotics can be based on many different microbials, such as *Bacillus*, lactic acid bacteria, yeast, etc. However, the *Bacillus*-based probiotics have proven to be the ideal candidate for in-feed applications, because they are resistant to heat and tolerant to acidic conditions. *Bacillus* occurs naturally in spore form, which contributes to their resiliency until they germinate in the gastrointestinal tract and grow as vegetative cells[19-20]. *Bacillus* spp., with soil as their natural residence are also used as probiotics, singularly or combined with lactic acidic bacteria or yeasts[19]. The supplementation of *Bacillus subtilis* (*B. subtilis*) in pig fattening feeds improved nutrient utilization, such as dry matter, which increased average daily gain (ADG) and meat quality[21-23].

The purposes of this study, therefore, were to assess the efficiency of two probiotics: 1. Sanizyme® (Quality Meat Co. LTD, Thailand) and 2. a commercial probiotic A and their supplemental effects on growth performance and nutrient digestibility in pig fattening diets.

MATERIALS AND METHODS

Animal care: The experimental procedures were approved by the Institutional Animal Care and Use Committee of Khon Kaen University (IACUC-KKU-73/60), Khon Kaen, Thailand.

Animals and diets: Experiments were conducted in a well-managed commercial farm (Mittraphap Farm) in Lopburi Province, Thailand. A total of 500 crossbred pigs ((Yorkshire- Landrace)×Duroc) with an average body weight (BW) of 22.70±1.2 kg (56-58 days of age), were divided into five treatments with four replications of 25 pigs each (two replications for males and two replications for females). All pigs were allotted treatments of completely randomized design (CRD) on the basis of gender, body weight and genetic background. The experimental dietary treatments consisted of the basal diet, the control group (T1), basal diet+*B. subtilis* (1.0×10^{12} CFU kg^{-1}) at level 0.1% (Pro A), (T2) and basal diet+*B. subtilis* (7.0×10^{9} CFU kg^{-1}) at levels 0.0125, 0.0250% and 0.0375% (Sanizyme®), represented as T3, T4 and T5, respectively, within the starter, grower and finisher diets. All pigs were housed in evaporation regulated, concrete floor fattening pens (5.0×7.0 m) throughout the study period (109 days). All pigs were given feed (in pellet form) and water *ad libitum* through one nine-hole feeder and two nipple water dispensers per pen. Pigs were weighed at each feed changing period (starter: 18 days, grower: 39 days and finisher: 52 days). The basal dietary formula consisted of broken rice, corn, rice bran and soybean meal, with a vitamin-mineral premix (formulated to meet National Research Council[24] recommendations). Feedstuff costs are presented in Table 1.

Sampling and measurements: Pigs were weighed at initial and final weights of the starter, grower and finisher periods. Body weights (BW) were recorded at the end of each period. Feed consumption was recorded per pen during the experiment to calculate body weight gain (BWG), average daily gain (ADG), average daily feed intake (ADFI), feed conversion ratio (FCR) and uniformity of body weight at termination (UBWT). Pig uniformity was represented as the percentage units that fell into two times of standard deviation based on average live weight at termination. Pigs were fed diets containing chromic oxide 0.2% (Cr_2O_3)[22], which were used as an indigestible marker, for seven days prior to fecal collection at the starter and grower periods for calculation of dry matter (DM), crude protein (CP), fat or ether extract (EE), crude fiber (CF) and gross energy (GE). Fecal grab samples

Table 1: Composition of experimental basal diets for starter (20-30 kg b.wt.), grower (30-60 kg b.wt.) and finisher (60-market weight) diets

	Content (%)		
Ingredients	Starter	Grower	Finisher
Broken rice (7.5% CP)	45.86-45.96	19.90-20.00	-
Corn (8% CP)	14.00	40.80	55.36-55.46
Rice bran (12.5% CP)	10.00	16.00	30.00
Soybean meal (46% CP)	19.00	16.40	9.00
Fish meal (60% CP)	5.00	2.00	1.50
Meat meal (50% CP)	5.00	2.00	1.50
Rice bran oil (unrefined)	2.00	1.30	-
DL-Methionine	0.09	0.07	0.02
L-lysine	0.24	0.16	0.22
Monocalcium phosphate	0.80	0.10	0.90
Calcium carbonate	-	0.30	0.60
Salt	0.19	0.27	0.30
Premixes[a]	0.50	0.50	0.50
Colistin 20%	0.06	0.05	-
Ammoxycilin 50%	0.06	0.05	-
Tiamulin 10%	0.10	-	-
Probiotics*	±	±	±
Cost, Baht kg^{-1}**	15.14	12.18	10.18
Composition by analysis			
CP (%)	19.02	17.01	14.45
GE (kcal kg^{-1})	4,056-4,095	4,122-4,185	4,150-4,170

[a]Premixes provide per kilogram of diet: A 2,500 IU, D_3 250 IU, E 20 IU, B_{12} 0.2 mg, Pantothenic acid 12 mg, Riboflavin 4 mg, Thiamine 2 mg, Choline chloride 1 g, K_3 0.5 mg, Biotin 0.3 mg, pyridoxine 2 mg, Folic acid 0.3 mg, Etoxyquia 12.5 mg, Cu 250 mg, Fe 100 mg, Zn 100 mg, Mn 4 mg, I 0.4 mg, Se 0.3 mg, Co 0.14 mg, Ammoxycilin 300 mg, Colistin 120 mg and Tiamulin 100 mg, *±Probiotics: Probiotics A = 0.1%, Sanizyme® = 0.0125, 0.0250 and 0.0375% **Probiotics: Probiotics A = 220 Baht kg^{-1}, Sanizyme® = 600 Baht kg^{-1}, Cost, Baht kg^{-1}: Starter; T1: 15.14, T2: 15.35, T3: 15.22, T4: 15.29 and T5: 15.36, Grower, T1: 12.18, T2: 12.39, T3: 12.25, T4: 12.33 and T5: 12.40, Finisher; T1: 10.18, T2: 10.39, T3: 10.25, T4: 10.33 and T5: 10.40

were obtained from each pen during the last three days of each period (starter: days 23-25, grower: days 44-46). All fecal and feed samples were stored in a freezer at -20°C until analyzed. Before chemical analysis, fecal samples were dried at 70°C for 72 h. All fecal samples, along with the feed samples, were analyzed for DM, CP, EE, CF and GE according to the AOAC procedures[25]. Chromium was analyzed via UV absorption spectrophotometry (T80+UV/VIS Spectrometer PG Instruments Ltd.) and the apparent total tract digestibility (ATTD) of DM, CP, EE, CF and GE were calculated according to the method described by Stein *et al.*[26].

Nutrients digestibility: Apparent digestibility coefficients for nutrients were calculated using the following Equation[26]:

$$\text{DMD (\%)} = \frac{\text{Indicator}_{\text{fecal}}(\%) - \text{Indicator}_{\text{feed}}(\%)}{\text{Indicator}_{\text{fecal}}(\%)} \times 100$$

where, DMD (%) is percentage of dry matter digestibility.

$$\text{AND (\%)} = 100 - \frac{\text{Indicator}_{\text{feed}}(\%) \times \text{Nutrient}_{\text{feces}}(\%)}{\text{Indicator}_{\text{feces}}(\%) \times \text{Nutrient}_{\text{feed}}(\%)} \times 100$$

where AND (%) is percentage of apparent nutrients digestibility.

Statistical analysis: Data for all response variables were analyzed through one-way analysis of variance, using the General Linear Model (GLM) procedure of the SPSS system[27]. Duncan's Multiple Range Test was used to determine treatment differences. The linear and quadratic contrasts compared the effects of increasing dietary *B. subtilis* levels (0, 0.0125, 0.0250 and 0.0375%). All statements of significance were based on the probability level of 0.05.

RESULTS

Growth performance: The effect of both brands of probiotics on growth performance in fattened pigs (starter: 18 days, grower: 39 days and finisher: 52 days) are shown in Table 2 and 3.

Starter period: Supplementation with both probiotics in this period showed improvement in BWG, ADG and FCR when compared with the control group, no significant effects were observed in BWG, ADG, ADFI and FCR when compared with the control group (p>0.05).

Grower and finisher period: Continuous feeding with both probiotics showed improvement in BWG, ADG and FCR when compared with the control group, no significant effects were observed in BWG, ADG, ADFI and FCR when compared with the control group (p>0.05).

During the overall experimental time frame, improvements were found in BWG, ADG, ADFI, FCR, UBWT and FCG, in comparison to the control group. However, feeding with both brands of probiotics within the overall time frame showed an increase in ADG and improvement in ADFI, FCR, UBWT and also showed decrease in FCG, which led to an increase in economic return, with 226.97 baht in T4, 203.76 baht in T2, 188.81 baht in T5 and 163.48 baht in T3, compared with the control group. The results from overall period showed that addition of Sanizyme® at the level of 25.0 g t^{-1} or 0.0250% showed the highest responses in growth performance and economic benefits return.

Digestibility evaluation: The effects of probiotic supplements on nutrient digestibility (DM, CP, EE, CF and GE) in the starter and grower periods are shown in Table 4 and 5. These results

Table 2: Effects of Sanizyme® additives in diets on growth performance in starter and grower periods

	CON[1]	Pro A[1]	Sanizyme® (SZ)[1]			Pooled
Items	T1	T2	T3	T4	T5	SEM
Initial number of pigs	100	100	100	100	100	
Final number of pigs	97	95	98	97	94	
Survival rate	97.00	95.00	98.00	97.00	94.00	
Initial weight (kg)	22.69	22.70	22.69	22.69	22.70	1.787
Final weight (kg)	98.53	101.24	100.33	101.60	100.76	6.581
Starter period, 18d Trial						
Av. Body weight gain (kg)	7.70	7.72	7.71	7.74	7.75	0.580
Av. Daily gain (g)	428	429	429	430	431	32.147
Av. Daily feed intake (g)	977	977	940	962	946	65.042
Feed: Gain (FCR)	2.289	2.297	2.195	2.243	2.202	0.174
Grower period, 39 d trial						
Av. Body weight gain (kg	30.87	31.16	31.08	31.13	31.29	1.934
Av. Daily gain (g)	792	799	797	798	803	49.526
Av. Daily feed intake (g)	1,902	1,847	1,851	1,861	1,828	138.923
Feed: Gain (FCR)	2.404	2.310	2.313	2.334	2.280	0.128

[1]Abbreviations: CON: T1, basal diet, Pro A: T2, (basal diet+*B. subtilis* at level 0.1%), SZ, (basal diet+*B. subtilis* at levels 0.0125, 0.0250 and 0.0375%) were representatives in T3, T4 and T5

Table 3: Effects of Sanizyme® additives in diets on growth performance in finisher and overall periods

	CON[1]	Pro A[1]	Sanizyme® (SZ)[1]			Pooled
Items	T1	T2	T3	T4	T5	SEM
Finisher period, 52 d trial						
Av. Body weight gain (kg)	37.27	39.66	38.85	40.04	39.02	4.270
Av. Daily gain (g)	717	763	747	770	751	82.204
Av. Daily feed intake (g)	2,459	2,446	2,456	2,473	2,430	257.497
Feed: Gain (FCR)	3.447	3.205	3.289	3.219	3.236	0.155
Overall period, 109 d trial						
Av. Body weight gain (kg)	75.84	78.54	77.64	78.91	78.06	5.481
Av. Daily gain (g)	696	720	712	724	716	50.322
Av. Daily feed intake (g)	2,015	1,989	1,989	2,004	1,970	178.659
Feed: Gain (FCR)	2.896	2.760	2.789	2.767	2.748	0.091
Uniformity of BW (%)	84.88	84.34	85.04	85.86	87.50	4.285
Feed cost/kg BWG, Baht	36.19	35.07	35.06	35.00	34.98	1.147
Av. Net profit/head, Baht*	3,322.31	3,526.07	3,485.79	3,549.28	3,511.12	221.086
Economic benefits return/head, baht**	+203.76	+203.76	+163.48	+226.97	+188.81	

[1]Abbreviations: CON: T1, basal diet; Pro A: T2, (basal diet+*B. subtilis* at level 0.1%); SZ, (basal diet+*B. subtilis* at levels 0.0125, 0.0250 and 0.0375%) were representatives in T3, T4 and T5, *Av. Net profit/head (Baht) = [(Average BWG, kg x Sale price of pigs, 80 Baht kg^{-1}) , -(Average BWG, kg x Feed cost/kg/BWG, Baht)], **Economic benefit return (Baht/head) = Average net profit (Treatment), -Average net profit (Control)

Table 4: Effects of Sanizyme® additives to diets on the apparent total tract digestibility of nutrients in the starter period

	CON[1]	Pro A[1]	Sanizyme®(SZ)[1]			Pooled	p-values[2]	
Items	T1	T2	T3	T4	T5	SEM	L	Q
DM	80.55[c]	82.60[b]	84.25[a]	83.23[ab]	82.22[b]	0.959	0.0545	0.0001
CP	71.25[b]	74.69[a]	76.09[a]	74.79[a]	74.10[a]	1.364	0.0137	0.0003
EE	78.13[b]	82.61[a]	83.67[a]	82.19[a]	82.82[a]	1.652	0.0041	0.0095
CF	36.13[b]	42.58[a]	43.78[a]	44.06[a]	42.31[a]	2.435	0.0039	0.0018
GE	81.76[c]	83.41[b]	85.25[a]	84.39[ab]	83.17[b]	0.917	0.0931	<0.0001

[a,b,c]Means in the same row with different superscripts differ significantly ($p<0.05$), [1] Abbreviations: CON: T1: Basal diet, Pro A: T2: (basal diet+*B. subtilis* at level 0.1%), SZ: (basal diet+*B. subtilis* at levels 0.0125, 0.0250 and 0.0375%) were representatives in T3, T4 and T5, [2]p-values for linear (L) and Quadratic (Q) effect for CON and SZ (0.0125, 0.0250 and 0.0375%)

demonstrate that both brands of additive probiotics in pigs diets displayed better ($p<0.05$) digestibility of nutrients (DM, CP, EE, CF and GE) in the diets of the starter and grower periods, when compared with the control group, whereas the

Table 5: Effects of Sanizyme® additives in diets on the apparent total tract digestibility of nutrients in the grower period

	CON[1]	Pro A[1]	Sanizyme®(SZ)[1]			Pooled	p-values[2]	
Items	T1	T2	T3	T4	T5	SEM	L	Q
DM	86.64[c]	90.40[a]	89.27[b]	88.74[b]	89.47[b]	0.578	<0.0001	0.0090
CP	83.03[c]	87.49[a]	85.76[b]	85.36[b]	86.20[ab]	0.895	0.0014	0.0818
EE	88.47	88.89	89.41	89.65	89.32	1.555	0.1815	0.1739
CF	49.02[b]	55.70[a]	58.40[a]	56.02[a]	56.71[a]	2.742	0.0030	0.0045
GE	88.14[c]	91.46[a]	90.46[b]	90.04[b]	90.65[b]	0.432	<0.0001	0.0030

[a,bc]Means in the same row with different superscripts differ significantly (p<0.05), [1] Abbreviations: CON: T1: Basal diet; Pro A: T2, (basal diet+*B. subtilis* at level 0.1%); SZ, (basal diet+*B. subtilis* at levels 0.0125, 0.0250 and 0.0375%) were representatives in T3, T4 and T5, [2]p-values for linear (L) and Quadratic (Q) effect for CON and SZ (0.0125, 0.0250 and0.0375%)

digestibility of EE in the grower period was of no significant difference (p>0.05). The addition of the probiotic Sanizyme® was present mostly on quadratic responses (p<0.05) on the digestibility of nutrients within the starter period. The grower period presented quadratic responses (p<0.05) in the diets representing DM, CF and GE and showed linear responses (p<0.05) in diets representing CP, when compared with the control group.

DISCUSSION

Growth performance: The results of the present study are supported by Alexopoulos *et al.*[28], who reported that a dietary supplementation of *Bacillus licheniformis*: *B. licheniformis* and *B. subtilis* spores in growing-finishing pigs showed significant (p<0.05) improvements in ADG and FCR parameters. According to Chen *et al.*[29] ADG increased significantly (p<0.05) with the addition of *B. subtilis*, *Lactobacillus acidophilus: L. acidophilus* and *Saccharomyces cerevisiae* in growing pig diets. Similarly, Chen *et al.*[30] also observed significant (p<0.05) improvements in ADG (p<0.05) when finishing pig fed diets included probiotics (*B. subtilis*, *Bacillus coagulans: B. coagulans* and *L. acidophilus*). Meng *et al.*[22] reported an increased ADG (p<0.01) in growing-finishing pig fed diets supplemented with complex probiotics (*B. subtilis* and *Clostridium butyricum*). According to Giang *et al.*[1] the supplementation of *B. subtilis* in the diet of growing-finishing pigs increased ADG (p<0.05) in the overall study period. Likewise, Upadhaya *et al.*[23] reported that growing-finishing pig fed diets supplemented with *B. licheniformis* and *B. subtilis* resulted in improved ADG (p<0.05) and overall ADFI (p = 0.06). Balasubramanian *et al.*[31] also observed a significant linear effect on ADG and Gain:Feed (p = 0.041, p = 0.019, respectively) in the overall results when growing-finishing pig fed diets included probiotics (*B. subtilis, B. coagulans* and *B. licheniformis*). Davis *et al.*[21] however, reported that the feed additives *B. licheniformis* and *B. subtilis* had no significant effects on the growth performance of growing-finishing pigs. Differences within the results of these studies may be explained by several factors, including the age of the pigs, strains of bacteria, the levels of probiotics, diet ingredients, feed form and interaction within the environment[32].

Digestibility evaluation: Chen *et al.*[29] observed that the digestibility of DM and N displayed a tendency to increase, whereas no significant differences were observed (p>0.05). Meng *et al.*[22] reported that the digestibilities of N and energy were significantly affected (p<0.05), yet the digestibility of DM was unaffected by the supplementation of probiotics. Likewise, Upadhaya *et al.*[23] suggested that digestibility of DM and N were significantly improved (p<0.05). However, according to Chen *et al.*[30] and Wang *et al.*[32] there were no significant effects on the digestibility of DM and N (p>0.05). More recently, Balasubramanian *et al.*[31]observed significant linear effects on nutrient digestibility of DM (p = 0.002) and a linear trend in N (p = 0.069) and energy (p = 0.099), at week 16. Kornegay and Risley[33] determined that *Bacillus* spp. in the form of a viable endospore preparation, maintain greater stability than vegetative cultures as reported in the diets of pigs and sows. Hong *et al.*[19] and Bermudez-Brito *et al.*[34] explained that the *Bacillus* species may inhibit the pathogen adhesion and competitive exclusion of pathogenic microorganisms, which may lead to an increase in the utilization of nutrients. Such improvements in nutrient digestibility in starter and grower periods may be due to the *Bacillus* species' secretion of enzymes, such as amylase, cellulose, lipase and protease[21].

CONCLUSION

In conclusion, both types of probiotic additives improve the utilization of nutrients (DM, CP, EE, CF and GE) in feed components in the starter and grower periods of the pig fattening diets as well as improved growth performance (ADG, ADFI, FCR and UBWT) and increased economic return, when compared with the control group.

SIGNIFICANCE STATEMENT

This study discover an alternative to antibiotic growth promoter for fattening pigs and found the possible effect of probiotic on nutrients digestibility, which led to improve growth performance and increase economic benefits. Therefore probiotics supplementation in the diet improve the growth performance and feed efficiency of fattening pigs. Supplementing probiotics in appropriated level may be a tool to improve the intestinal microflora as this reduces the number of pathogenic bacteria in the gastrointestinal tract in fattening pigs.

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