The effects of using multi-species probiotics in late-pregnant and lactating sows on milk quality and quantity, fecal microflora, and performance of their offspring

Narathon Innamma¹, Natharin Ngamwongsatit^{1,2}, and Kampon Kaeoket¹

 Department of Clinical Sciences and Public Health, Faculty of Veterinary Science, Mahidol University, Nakhon Pathom, 73170, Thailand;
 Laboratory of Bacteria, Veterinary Diagnostic Center, Faculty of Veterinary Science, Mahidol University, Nakhon Pathom, 73170, Thailand.

Corresponding author: Kampon Kaeoket, e-mail: kampon.kae@mahidol.edu
Co-authors: NI: narathon.inn@student.mahidol.edu, NN: natharin.nga@mahidol.edu
Received: 11-06-2023, Accepted: 13-09-2023, Published online: 07-10-2023

doi: www.doi.org/10.14202/vetworld.2023.2055-2062 **How to cite this article:** Innamma N, Ngamwongsatit N, and Kaeoket K (2023) The effects of using multi-species probiotics in late-pregnant and lactating sows on milk quality and quantity, fecal microflora, and performance of their offspring, *Veterinary World*, 16(10): 2055–2062.

Abstract

Background and Aim: The dietary probiotics in sows during gestation to lactation period have gained considerable attention with respect to their beneficial effects on sows and their piglets' performance and health. This study aimed to evaluate the effects of using probiotics in late-pregnant and lactating sows on milk quality, quantity, fecal microflora of sows, and growth performance of their offspring until weaning.

Materials and Methods: Thirty-four sows were equally divided into two groups (control and treatment). Only those in the treatment group were fed 5 g of probiotics at 12 weeks of pregnancy, once daily for 7 weeks, until their piglets were weaned. Colostrum samples were collected at 3, 6, 12, and 24 h after farrowing and measured for immunoglobulin concentration. Percentages of fat, protein, and lactose in colostrum, colostrum production, total intake of immunoglobulin A (IgA), immunoglobulin G (IgG), fat, protein, and lactose, the change of fecal microflora of sows, and average daily gain of piglets were measured.

Results: The results showed that there were no significant differences in the concentrations of IgA, IgG, and IgM in colostrum and the percentages of fat, protein, lactose, solid-not-fat, and total solid in colostrum between the groups; however, the colostrum production at 24 h in the treatment group (6,075.29 mL) was higher than in the control group (4,809.54 mL). Higher total intakes of IgA and IgG as well as total intake of fat, protein, and lactose, particularly at 3 h after farrowing, were found in the treatment group. Probiotic supplementation remarkably altered the microbiota community at the phylum level. We found that Firmicutes and Bacteroidetes are the dominant phyla, present in the gut of more than 90% of pregnant and lactating sows. Changes in microbial proportions were observed due to the changes of pig production stage. The weaning weight of the treatment group was higher than in the control group $(6.34 \pm 1.71 \text{ vs. } 4.84 \pm 1.29 \text{ kg, respectively})$.

Conclusion: Feeding of multi-species probiotic BACTOSAC-PTM during late pregnancy and lactation in sows positively influenced colostrum production. In this experiment, the use of BACTOSAC-PTM improved the yield of colostrum production. The high immunoglobulin concentration and high yield of the colostrum of sows with a diet supplemented with BACTOSAC-PTM significantly reduced piglet mortality during the suckling period. Furthermore, the probiotic diet induced changes in the fecal microbial population in sows by increasing the number of microorganisms from the Firmicutes phylum, which had positive effects on sow health and their piglets, leading to better piglet growth performance.

Keywords: fecal microflora, lactation, probiotics, swine, weaning.

Introduction

Pigs are one of the major forms of livestock in Asian countries, including Thailand. They are raised in intensive housing environments, making them more susceptible to infectious diseases. Antibiotics are extensively used in pigs as growth promoters and preventive medicines. However, the increased use of antibiotics leads to an increase in antibiotic-resistant

Copyright: Innamma, et al. Open Access. This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated.

bacterial strains, which can harm both humans and animals [1]. It has been suggested that a colistin-resistance gene (mcr-1) found in Escherichia coli originating from pigs may be transferred directly or indirectly to humans [2]. In pig-producing countries globally, including Thailand, increasing attention is being paid to reducing the use of antibiotics for preventive purposes, with only clinical application for treating sick animals being allowed. Unfortunately, this reduced use of antibiotics has caused an increase in bacterial infection in pigs. The associated morbidity and mortality rates in the weaning period have been reported to be high [3]. Therefore, the replacement of antibiotics with an alternative to reduce the losses of pigs and not facilitate antibiotic resistance has been pursued. Such alternatives to replace antimicrobials

include antibacterial vaccines, immunomodulatory agents, bacteriophages, antimicrobial peptides, probiotics, prebiotics, synbiotics, plant extracts, and feed enzymes [4].

Probiotics are living microorganisms that promote host gut health. They confer benefits such as maintaining homeostasis in the gastrointestinal (GI) tract, and improving digestion and the overall health of the host [5]. In the last decade, many studies have reported on the use of probiotics in pigs, for example, using Lactobacillus amylovorus and Enterococcus faecium, Lactobacillus reuteri, Bacillus licheniformis, and *Bacillus subtilis* to establish good gut health [6–8]. It has also been recently reported that some probiotics showed an inhibitory effect on pig bacterial pathogens [9, 10], subsequently leading to higher average daily gain (ADG), lower feed conversion ratio (FCR), and improved physiological conditions [11]. Probiotics in the GI tract also inhibits colonization by other pathogens, preventing them from establishing themselves in the animal's body. Moreover, lactic acid-producing bacteria (LAB), such as Lactobacillus spp., can create acidic environments unsuitable for particular pathogens [9]. Besides, probiotics can also stimulate the host immune system by modulating tolllike receptors (TLRs) on cell membranes of the GI tract and signaling the production of immunostimulant cytokines [12]. In newborn piglets, colostrum, which is high in immunoglobulin G (IgG) and immunoglobulin A (IgA) content, can promote health, growth, intestinal growth and function, and immunity of piglets [13–15]. It can also provide sufficient energy for thermoregulation during the first 24 h of life, which is critical for the survival of piglets. It was reported that piglets with colostrum consumption of 250 g could achieve good health and growth [16, 17]. The gut microbiota composition of sows during pregnancy and lactation can impact enteric nutrient absorption and immunity [18], which influences the body weight (BW) of piglets at birth and weaning, the number of piglets born alive, the rate of pre-weaning mortality, and the number of piglets weaned per litter [19]. It has been documented that adding a single strain of probiotics (B. licheniformis or B. subtilis spores) in pig feed could increase intake during lactation in sows, prevent body mass loss, improve colostrum content (i.e., immunoglobulin content, fat, protein, lactose, solid-not-fat [SNF], and total solid [TS]), decrease the occurrence of diarrhea in piglets, lower the pre-weaning mortality rate, and increase the weaning weight [20]. Nonetheless, little information has been reported on the effects of multi-species probiotics on quantity and quality of the colostrum of sows and the performance of their offspring.

Therefore, we studied the effects of using a multi-species probiotic product in late-pregnant and lactating sows on milk quality and quantity, the changes in fecal microflora of sows, and the performance of their offspring.

Materials and Methods

Ethical approval

This research project was approved by the Faculty of Veterinary Science, Mahidol University-Institute Animal Care and Use Committee (MUVS-2018-06-26).

Study period and location

This study was conducted from October 2018 to December 2019 on a private farrow-to-finish pig farm with an open house system in Chonburi province, Thailand. The farm was with approximately 3,000 sows.

Probiotics

multi-species probiotic The product (BACTOSAC-PTM: K.M.P. Biotech Co., Ltd., Thailand) used in this study is composed of seven living strains of different bacteria at different concentrations (Table-1). According to a molecular test performed at the Department of Microbiology, Faculty of Medicine Siriraj Hospital, Mahidol University, none of these probiotic strains carried an antibiotic resistance gene (unpublished data). The recommended dose of these probiotics was 5 g per meal sprinkled on top of the feed, once daily from week 12 of pregnancy until weaning of sows, for 7 weeks in total.

Experimental animals

Overall, 34 Landrace × Yorkshire primiparous and multiparous sows (at 12 weeks of pregnancy) were randomly divided into two groups: Control group (n = 17 sows) and treatment group (n = 17 sows, fed with BACTOSAC-PTM powder). They were kept in an open-housing system, fed with farm mixed feed at 5–6 kg/day, and provided with water *ad libitum*. In the treatment group, sows were fed a standard diet supplemented with probiotic at 5 g/day starting on day 84 of gestation until the end of the 24-day lactation period. Meanwhile, in the control group, sows were fed a standard diet for late gestation and lactating sows until the end of the 24-day lactation period.

Data collection

At least 16 mL of sow colostrum was collected in both groups by hand milking [21] at 3, 6, 12, and 24 h after farrowing from four to six teats in the anterior part of the udder. Colostrum samples were kept at 4°C

Table-1: Composition of multi-species probiotic product (BACTOSAC- P^{TM}).

	Microbial composition	Content per gram of product
1.	Lactobacillus acidophilus	1.0×10 ⁷ CFU/g
2.	Lactobacillus plantarum	1.0×10 ⁷ CFU/g
3.	Enterococcus faecium	1.0×10 ⁷ CFU/g
4.	Pediococcus pentosaceus	1.0×10 ⁶ CFU/g
5.	Bacillus subtilis	1.0×10 ⁷ CFU/g
6.	Bacillus licheniformis	1.0×10 ⁷ CFU/g
7.	Saccharomyces cerevisiae	1.0×10 ⁶ CFU/g

CFU=Colony-forming unit

during transportation to the laboratory and stored at -20°C until further analysis of milk composition and immunoglobulins.

Before analysis, the frozen samples were thawed at room temperature (28°C–30°C). Three milliliters of colostrum was used to measure the level of immunoglobulin using Pig Ig enzyme-linked immunoassay test kits, in accordance with the manufacturer's instructions (Koma Biotech, Seoul, Republic of Korea). Thirteen milliliters of colostrum was used to analyze milk composition (i.e., % fat, % protein, % lactose, SNF, and TS) by MilkoscanTM FT1 (Foss Electric, Hillerød, Denmark) [22, 23].

Sow colostrum production

Sow colostrum production was indirectly calculated from the piglet BW gain at 24 h after farrowing and transformed into the colostrum intake (CI) using the following equation of Theil *et al.* [24]:

CI(g) = -106 + 2.26 WG + 200 BWB + 0.111D- 1,414 WG/D + 0.0182 WG/BWB

Where CI = Colostrum intake (g), WI = 24 h weight gain of piglet (g), BWB = Pig body weight at birth (kg), and D = Time elapsed from birth to weighing at t_{24} (min).

Calculation of total intake of IgA, IgG, protein, fat, and lactose

The total intake of IgA and IgG at 3, 6, and 12 h was calculated as follows:

Total intake of Ig = concentration of $Ig \times (colostrum production at 24 h/average number of piglets born alive in each group).$

The total protein, fat, and lactose intake was also calculated using the same equation.

Growth performance of piglets

The ADG of piglets in each group was calculated as follows: ADG = $(BW_{wean} - BW_{birth})/Day$

Where ADG = Average daily gain (g/day), BW_{wean} = Pig body weight at weaning (kg), BW_{birth} = Pig body weight at birth (kg), and Day = Nursing period (day).

Microbiota diversity analysis

Pooled samples of feces were collected from sows in both groups at week 12 of pregnancy and at 1 week after farrowing. Total DNA was extracted from fecal samples using QIAamp Stool DNA Extraction kit (QIAGEN, Germany). 16s rRNA was amplified from each pooled DNA sample and then sequenced using the Illumina MiSeq platform (San Diego, CA, USA). Overlapping paired-end reads were assembled using PEAR. The reads were processed using Quantitative Insights into Microbial Ecology v1.9.0 [25] for operational taxonomic units picking and taxonomy.

Statistical analysis

Data on colostrum quality and quantity, and performance of the offspring were analyzed using the general linear model from IBM Statistical Package for the Social Sciences Software version 22.0 (IBM Corporation, New York, USA). The t-test was

employed to compare the volume of colostrum and composition (i.e., fat, protein, lactose, SNF, and TS), piglet performance, and immunoglobulin content (i.e., IgA, IgM, and IgG). All data are presented as mean \pm standard deviation and the differences between the results of the treatment and control groups were considered statistically significant at p \leq 0.05.

Results

Milk quality and quantity

The immunoglobulin levels (i.e., pig IgG, IgA, and IgM) and colostrum composition in the control and treatment groups are shown in Tables-1 and 2, respectively. The two groups had no significant difference in the immunoglobulin levels (p > 0.05). The IgA level in the treatment group peaked at 12 h $(17.46 \pm 5.30 \text{ mg/mL})$, while the peak in the control group occurred at 6 h (20.51 \pm 6.20 mg/mL). Regarding the IgG level in the control and treatment groups, their peaks were found at 3 h post-farrowing $(198.27 \pm 79.66 \text{ vs. } 179.74 \pm 62.07 \text{ mg/mL})$ and gradually decreased over time. Unlike IgA and IgG levels, the IgM levels were lower than 10 mg/mL over time. In the milk composition analysis using MilkoscanTM FT1, there were no significant differences in terms of percentages of fat, protein, lactose, SNF, and TS. Fat and protein percentages gradually decreased over time, while the percentage of lactose tended to plateau.

Sow colostrum production and piglet performance

The calculated sow colostrum production revealed higher colostrum production in the treatment group than in the control group $(6,075.29 \pm 1,419.46 \text{ g/L})$ and $4,809.54 \pm 616.49 \text{ g/L})$. Therefore, we calculated the average piglet CI at 24 h using the number of piglets born alive in both treatment (13.5 piglets/L) and control (14.0 piglets/L) groups. The results revealed a higher CI in the treatment group than in the control group $(450.02 \pm 105.15 \text{ g/piglet vs.} 343.54 \pm 44.04 \text{ g/piglet})$. This resulted in a higher total intake of IgA, IgG, fat, protein, and lactose by each piglets at 3, 6, and 12 h in the treatment group than in the control group, as shown in Tables-3 and

Table-2: Concentration of immunoglobulin (mg/mL) in colostrum at 3, 6, and 12 h after farrowing in control and treatment groups.

Parameters	Control	Treatment	p-value
IgG	-		
3 h	198.27 ± 79.66	179.74 ± 62.07	0.91
6 h	189.40 ± 77.08	164.85 ± 90.42	0.71
12 h	158.59 ± 26.85	163.61 ± 121.08	0.52
IgA			
3 h	18.62 ± 5.16	16.36 ± 6.97	0.93
6 h	20.51 ± 6.20	16.87 ± 6.95	0.36
12 h	17.91 ± 4.78	17.46 ± 5.30	0.91
IgM			
3 h	4.75 ± 1.21	7.35 ± 3.30	0.33
6 h	5.11 ± 1.34	4.93 ± 1.27	0.89
12 h	4.42 ± 0.86	4.05 ± 1.27	0.94

IgG=Immunoglobulin G, IgA=Immunoglobulin A, IgM=Immunoglobulin M

Table-4. Regarding the growth performance of piglets, ADG was significantly higher in the treatment group $(176.0 \pm 63.22 \text{ g/day}; n = 201)$ than in the control group $(121.0 \pm 47.23 \text{ g/day}; n = 209)$ (p < 0.05). On comparing the pre-weaning mortality rate, this rate was lower in the treatment group (10.95%) than in the control group (14.83%).

Changes of sow fecal microbial diversity

The results of genomic (DNA) sequencing at the phylum level are presented in Figure-1. A total of 16 phyla were found in the fecal samples of the sows in the 12th week of pregnancy. At the phylum level, Firmicutes and Bacteroidetes were dominant taxa of more than 80% of the total sequences when

Table-3: Percentages of fat, protein, lactose, SNF and TS in colostrum at 3, 6, and 12 h after farrowing in control and treatment groups.

Colostrum composition	Control	Treatment	p-value
Fat			
3 h	7.94 ± 0.73	7.56 ± 1.25	0.66
6 h	9.51 ± 1.48	6.53 ± 1.97	0.00
12 h	7.88 ± 1.34	5.82 ± 1.82	NA
Protein			
3 h	13.28 ± 0.49	15.51 ± 2.42	0.18
6 h	15.43 ± 1.97	14.91 ± 1.97	0.71
12 h	14.95 ± 1.67	13.59 ± 2.30	NA
Lactose			
3 h	3.32 ± 0.40	2.98 ± 0.40	0.29
6 h	2.81 ± 0.67	2.99 ± 0.39	0.59
12 h	3.73 ± 0.61	3.39 ± 0.43	NA
SNF			
3 h	16.74 ± 0.11	18.40 ± 1.81	0.19
6 h	18.2 ± 1.27	17.87 ± 1.46	0.75
12 h	18.55 ± 1.14	17.06 ± 1.72	NA
TS			
3 h	26.09 ± 0.71	27.63 ± 2.98	0.43
6 h	29.42 ± 2.96	26.06 ± 2.94	0.13
12 h	28.08 ± 2.52	24.36 ± 3.16	NA

NA=Not analyses, SNF=Solid not fat, TS=Total solid

Table-4: The total intake by calculation of fat, protein, lactose, SNF, and TS by piglets during 12 h in control and treatment groups.

a calculation graduation					
Hours	Control	Treatment			
	343.54	405.02			
3 h	68,113.68	72,798.30			
6 h	65,066.48	66,767.55			
12 h	54,482.01	66,265.32			
3 h	6,396.72	6,626.13			
6 h	7,046.01	6,832.69			
12 h	6,152.80	7,071.65			
3 h	27.28	30.62			
6 h	32.67	26.45			
12 h	27.07	23.57			
3 h	45.62	62.82			
6 h	53.01	60.39			
12 h	51.36	55.04			
3 h	11.41	12.07			
6 h	9.65	12.11			
12 h	12.81	13.73			
	3 h 6 h 12 h 3 h 6 h 12 h 3 h 6 h 12 h 3 h 6 h	343.54 3 h 68,113.68 6 h 65,066.48 12 h 54,482.01 3 h 6,396.72 6 h 7,046.01 12 h 6,152.80 3 h 27.28 6 h 32.67 12 h 27.07 3 h 45.62 6 h 53.01 12 h 51.36 3 h 11.41 6 h 9.65			

SNF=Solid not fat, TS=Total solid, IgG=Immunoglobulin G, IgA=Immunoglobulin A

compared with other phylum, Firmicutes for more than 79.32% and 80.94%, and Bacteroidetes for approximately 10.59% and 9.29% in control and treatment groups, respectively. As shown in Figure-2. the percentages of dominant phyla of gut microbes at 1 week of lactation in pregnant sows in the control group were as follows: 75.04% for Firmicutes, 9.39% for Proteobacteria, 7.88% for Bacteroides, and 4.14% for Spirochaetes. Meanwhile, in the treatment group, the proportions were 80.25% for Firmicutes, 6.62% for Bacteroides, 5.51% for Proteobacteria, and 3.83% for Spirochaetes. The microbial diversity analysis showed shifts in the gut microbial diversity only in sows in the control group. The results showed that the levels of Firmicutes and Bacteroidetes decreased during the lactation period.

Discussion

Taking the findings together, it was suggested that feeding pregnant sows with multi-species probiotics from week 12 of pregnancy over a 7-week-period benefits milk quality and quantity and the growth performance and pre-weaning mortality of their offspring. Many mechanisms can be proposed to explain the obtained results. For example, providing probiotics has been proven in many studies to improve the balance in gut microbiota, the production of volatile fatty acids and vitamins, and the development of the immune system [26]. Besides, it has been demonstrated that probiotic feeding can increase feed

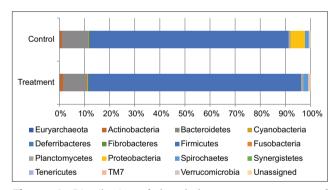


Figure-1: Distribution of the phylum as a percentage of the total number of identified 16s rRNA sequences from fecal samples of the 12th week pregnant sows.

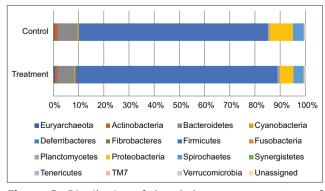


Figure-2: Distribution of the phylum as a percentage of the total number of identified 16s rRNA sequences from fecal samples of the 1st week lactating sows.

consumption and nutrient utilization in sows during pregnancy and lactation [20]. This may explain the superior colostrum production of sows in the treatment group in this study.

It is well documented that probiotics contain various microbiota strains and benefit the animal's overall health. Recently, in vitro and in vivo findings have been reported describing that LAB and Bacillus spp. can inhibit the growth of pathogenic bacteria from pigs [9, 10] and improve gut health and performance of nursing and finishing pigs in an experimental study [20]. Particular Lactobacillus species have the ability to prevent the colonization of pathogens through competitive exclusion and promotion of the production of short-chain fatty acids (acetic acid and propionic acid), which can suppress pathogens by creating an acidic environment [27]. They also enable nutrients that are not normally digestible by the host to be digested and absorbed, hence helping to improve the health status and growth of the weaned pigs [28]. Lactobacillus and Pediococcus strains can enhance the antioxidative defense system of weanling pigs and consequently prevent intestinal infections caused by enterotoxigenic E. coli. Bacillus probiotics positively affect pigs, such as higher weight gain, improved FCRs, and reduced incidence of diarrhea and mortality in piglets [29]. This may support the present results in that feeding on multi-strain probiotics could regulate gut health by modulating bacterial communities in the intestinal tract, improving feed efficiency and growth rate, enhancing the production of short-chain fatty acids, and inhibiting pathogens in the animals [28]. However, it is worth noting that the effects of probiotics could vary depending on the particular strains of microbiota contained within them. The multi-strain probiotics used in this study contained Lactobacillus spp., Pediococcus spp., Bacillus spp., Enterococcus spp., and Saccharomyces spp., which could have multiple functions and benefits for all of the sows and their piglets.

A previous study by Zhang et al. [8] in pigs showed that probiotics can modulate the immune system by regulating immune cell activation and increasing antibody production. Toll-like receptors are among the key recognition receptors in the innate immune system. Increased TLR expression releases pro- and anti-inflammatory cytokines when probiotics are used to stimulate the innate immune system. It has also been reported that Bacillus amyloliquefaciens SC06 alleviated the intestinal inflammation of fattening pigs by regulating the expression of proinflammatory cytokines, including interleukin (IL)-6, IL-8, and monocyte chemotactic protein 1 [30]. Besides Bacillus, Lactobacillus fermentum and Pediococcus acidilactici also reduced the concentration of the serum proinflammatory cytokines IL-6 and interferon-y in the serum of weaned piglets and helped to reduce the damage caused by inflammation [28]. Moreover, Laskowska et al. [31] showed

high levels of pro- and anti-inflammatory cytokines in serum of pregnant sows, indicating that probiotic supplementation has an immunomodulatory effect on systemic immune processes, manifested in part as improvement in the immune quality of the colostrum. The use of multimicrobial probiotic formulation products as dietary supplements in sows increased the concentrations of the proinflammatory cytokines tumor necrosis factor-α and IL-6, which increased the protective capacity of the colostrum by stimulating immune cell mechanisms protecting the sows and their piglets against infection. Besides, the results from study of Laskowska et al. [31] showed increased concentrations of anti-inflammatory cytokines IL-4, IL-10, and transforming growth factor-β, as well as IgG and IgA in the colostrum and milk from sows in the experimental group. This indicated the immunoregulatory effect of multimicrobial probiotics on Th2 cells and increased regulatory T cell expression. These findings correspond to the results of previous studies by Laskowska et al. [31], Jarosz et al. [32], and Tsukahara et al. [33], which show that including probiotics in animal diets during pregnancy and lactation increased the immune potential of colostrum and milk and protected against infectious diseases. In this study, although there was no significant difference in the IgG and IgA concentrations in sow colostrum between the groups, the greater production of colostrum and milk by sows in the probiotic-supplemented group resulted in a higher intake of colostrum by the piglets during the first 24 h of life. The piglets of the sows given probiotics consumed more colostrum at 24 h after farrowing (309.3 g/piglet), so they consumed more total fat, total protein, total lactose, and total immunoglobulins (i.e., IgA and IgG), resulting in higher growth performance and lower pre-weaning mortality than in the control group. This is in agreement with the previous studies by Nuntapaitoon et al. [16] and Nuntapaitoon et al. [17], in which it was proposed to achieve good health in pre- and post-weaning piglets, 250 g of colostrum should be consumed in the first 24 h after birth, with a minimum of 200 g/piglet. Furthermore, positive correlations have been identified between increased CI and decreased mortality rate, and a subsequent long-term increase in piglet growth [32]. This would have been due to the higher levels of immunoglobulin, fat, protein, and energy transferred to the piglets, which are essential for piglet survival [20, 32–36]. The use of BACTOSAC-PTM during pregnancy and lactation positively affected growth performance and piglet health. The high level of immunoglobulins in colostrum of the sows whose daily diets were supplemented with BACTOSAC-PTM significantly reduced piglet mortality. This might be explained by the probiotics exerting immunomodulatory effects, increasing secretory IgA and secretory IgG in colostrum/milk, which, in turn, prevented infection by pathogenic bacteria in the GI tract of piglets. This would subsequently influence piglet performance and

reduce pre-weaning mortality at this particular pig farm. In agreement with the present results, the piglets in the treatment group showed significantly higher ADG than the piglets in the control group, which was attributed to the higher amount of colostrum consumed, so more passive immunity was received and better growth subsequently occurred [31, 32, 35].

The ecosystem of the GI tract is complex and plays an important role in both promoting health and preventing disease. A number of studies have investigated the metagenomic characteristics of the intestinal microbiota after probiotic supplementation in pig [37–39]. An increased level of LAB was mainly observed in the analysis of the microbiota of suckling piglets after supplementation with Lactobacillus probiotics. The microbiome of the intestinal tract of pig undergoes a post-weaning transition where lactobacilli dominate the intestinal microbiota of suckling piglets, while members of Firmicutes and Bacteroides are predominant in adult pigs [38, 39]. During the weaning process, lactobacilli populations were detected at significantly lower levels after exposure to stress factors, suboptimal feed intake, and transportation [39]. Furthermore, a shift in composition and activities of the predominant microbiota, and emergence of clostridia and E. coli, were encountered in the intestinal tract of the piglets early in the post-weaning stage [40]. However, it has been indicated that the supplementation of probiotics can reduce the number of pathogenic bacteria in the gut while increasing the relative abundance of commensal lactobacilli and bifidobacteria [41]. Many studies have shown that probiotic supplementation could help to balance the bacterial community [39–41], which may also promote the health of sows. In agreement with our study, probiotic supplementation has been reported to induce remarkable alterations in the microbiota community at the phylum level. We found that Firmicutes and Bacteroidetes are the dominant phyla present in the gut of more than 90% of pregnant and lactating sows. Changes in microbial proportions were observed in this study, which might be due to the changes of pig production stage. The ratio of these two phyla in the digestive tract can affect the capacity to absorb nutrients from ingested feed. It has also been reported that body fat percentage is positively correlated with the abundance of Firmicutes in the gut microbiota [42]. Cui et al. [43] demonstrated that B. subtilis probiotics decreased the number and percentage of Bacteroides and increased the percentage of Firmicutes in cecal contents. Furthermore, pigs receiving probiotic supplementation had higher backfat thickness than control pigs. Backfat thickness is one of the significant parameters of female pigs correlated with reproductive performance, for example, puberty attainment, total piglets born, and farrowing rate. Besides, backfat is one of the significant sources of hormones related to puberty attainment, such as leptin, insulin-like growth factor-I, and progesterone (P4) [44].

Conclusion

The feeding of sows on multi-species probiotics (BACTOSAC-PTM) during late pregnancy and lactation positively affected colostrum production. In this experiment, the use of BACTOSAC-PTM improved colostrum yield in sows by improving the digestibility of dietary nutrients and overall feed efficiency owing to the production and activity of digestive enzymes by Bacillus spp. contained in the probiotic. Furthermore, the probiotic type used in this study is composed of a variety of microbial probiotics, for example, Lactobacillus spp., Pediococcus spp., Enterococcus spp., Bacillus spp., and Saccharomyces spp., which have multiple functions in promoting colostrum production and immunoglobulin production in colostrum/milk, reducing piglet mortality, achieving better growth performance results, and reducing financial losses of farms.

Authors' Contributions

KK: Study conception and design. NI, NN, and KK: Conducted experiments and wrote the manuscript. NI and KK: Analyzed data. All authors have read, reviewed, and approved the manuscript.

Acknowledgments

The authors are thankful to Mahidol University, National Research Council of Thailand (NRCT, grant numbers 1059, 3728, and 17553) and K.M.P. Biotech (Thailand) for funding the study. We are gratefully thankful for those people who have participated in our experiment. We also appreciate Ms. Suwanna Sanyutitham for providing laboratory facilities and equipment at the Veterinary Medical Center for Livestock and Wildlife Animal Hospital, Kanchanaburi.

Competing Interests

The authors declare that they have no competing interests.

Publisher's Note

Veterinary World remains neutral with regard to jurisdictional claims in published institutional affiliation.

References

- Nguyet, L.T.Y., Keeratikunakorn, K., Kaeoket, K. and Ngamwongsatit, N. (2022) Antibiotic resistant *Escherichia* coli from diarrheic piglets from pig farms in Thailand that harbor colistin-resistant mcr genes. *Sci. Rep.*, 12(1): 9083.
- Hershberger, E., Oprea, S.F., Donabedian, S.M., Perri, M., Bozigar, P., Bartlett, P. and Zervos, M.J. (2005) Epidemiology of antimicrobial resistance in enterococci of animal origin. J. Antimicrob. Chemother., 55(1): 127–130.
- Diana, A., Boyle, L.A., Leonard, F.C., Carroll, C., Sheehan, E., Murphy, D. and Manzanilla, E.G. (2019) Removing prophylactic antibiotics from pig feed: How does it affect their performance and health? *BMC Vet. Res.*, 15(1): 67.
- Cheng, G., Hao, H., Xie, S., Wang, X., Dai, M., Huang, L. and Yuan, Z. (2014) Antibiotic alternatives: The substitution of antibiotics in animal husbandry? *Front. Microb.*, 5:217.

- Liao, S.L. and Nyachoti, M. (2017) Using probiotics to improve swine gut health and nutrient utilization. *Anim. Nutr.*, 3(4): 331–343.
- 6. Hou, C., Zeng, X., Yang, F., Liu, H. and Qiao, S. (2015) Study and use of the probiotic *Lactobacillus reuteri* in pigs: A review. *J. Anim. Sci. Biotechnol.*, 6(1): 14.
- Zhu, C., Yao, J., Zhu, M., Zhu, C., Yuan, L., Li, Z. and Hu, P. (2022) A meta-analysis of *Lactobacillus*-based probiotics for growth performance and intestinal morphology in piglets. *Front. Vet. Sci.*, 9: 1045965.
- 8. Zhang, Y., Zhang, Y., Liu, F., Mao, Y., Zhang, Y., Zeng, H. and Yu, J. (2023) Mechanisms and applications of probiotics in prevention and treatment of swine diseases. *Porcine Health Manag.*, 9(1): 5.
- Kaewchomphunuch, T., Charoenpichitnunt, T., Thongbaiyai, V., Ngamwongsatit, N. and Kaeoket, K. (2022) Cell-free culture supernatants of *Lactobacillus* spp. and *Pediococcus* spp. inhibit growth of pathogenic *Escherichia coli* isolated from pigs in Thailand. *BMC Vet. Res.*, 18(1): 60.
- Keeratikunakorn, K., Kaewchomphunuch, T., Kaeoket, K. and Ngamwongsatit, N. (2023) Antimicrobial activity of cell free supernatants from probiotics inhibits against pathogenic bacteria isolated from fresh boar semen. Sci. Rep., 13(1): 5995.
- Pereira, W.A., Franco, S.M., Reis, I.L., Mendonça, C.M.N., Piazentin, A.C.M., Azevedo, P.O.S. and Oliveira, R.P.S. (2022) Beneficial effects of probiotics on the pig production cycle: An overview of clinical impacts and performance. *Vet. Microbiol.*, 269: 109431.
- Ng, S.C., Hart, A.L., Kamm, M.A., Stagg, A.J. and Knight, S.C. (2009) Mechanisms of action of probiotics: Recent advances. *Inflamm. Bowel. Dis.*, 15(2): 300–310.
- Nuntapaitoon, M. (2022) Colostrum and milk in sow. In: Milk Protein-new Research Approaches. IntechOpen, United Kingdom.
- 14. Tan, C., Zhai, Z., Ni, X., Wang, H., Ji, Y., Tang, T. and Yin, Y. (2018) Metabolomic profiles reveal potential factors that correlate with lactation performance in sow milk. *Sci. Rep.*, 8(1): 10712.
- Ferrari, C.V., Sbardella, P.E., Bernardi, M.L., Coutinho, M.L., Vaz, I.S. Jr., Wentz, I. and Bortolozzo, F.P. (2014) Effect of birth weight and colostrum intake on mortality and performance of piglets after cross-fostering in sows of different parities. *Prev. Vet. Med.*, 114(3–4): 259–266.
- Nuntapaitoon, M., Juthamanee, P., Theil, P.K. and Tummaruk, P. (2020) Impact of sow parity on yield and composition of colostrum and milk in Danish landrace × Yorkshire crossbred sows. *Prev. Vet. Med.*, 181: 105085.
- Nuntapaitoon, M., Muns, R., Theil, P.K. and Tummaruk, P. (2019) Factors influencing colostrum consumption by piglets and their relationship with survival and growth in tropical climates. *Livest. Sci.*, 224: 31–39.
- Sekirov, I., Russell, S.L., Antunes, L.C.M. and Finlay, B.B. (2010) Gut microbiota in health and disease. *Physiol. Rev.*, 90(3): 859–904.
- 19. Declerck, I., Dewulf, J., Sarrazin, S. and Maes, D. (2016) Long-term effects of colostrum intake in piglet mortality and performance. *J. Anim. Sci.*, 94(4): 1633–1643.
- Alexopoulos, C., Georgoulakis, I.E., Tzivara, A., Kritas, S.K., Siochu, A. and Kyriakis, S.C. (2004) Field evaluation of the efficacy of a probiotic containing *Bacillus licheniformis* and *Bacillus subtilis* spores, on the health status and performance of sows and their litters. *J. Anim. Physiol. Anim. Nutr.* (Berl), 88(11–12): 381–392.
- 21. Quesnel, H. (2011) Colostrum production by sows: Variability of colostrum yield and immunoglobulin G concentrations. *Animal*, 5(10): 1546–1553.
- Rolinec, M., Biro, D., Galik, B., Simko, M., Juracek, M., Kanka, T. and Schubertova, Z. (2012) Changes in the nutrient content of colostrum of sows during parturition. *Res. Pig Breed*, 6(2): 62–66.
- 23. Skrzypczak, E., Waśkiewicz, A., Beszterda, M., Goliński, P.,

- Szulc, K., Buczyński, J.T. and Babicz, M. (2015) Impact of fat and selected profiles of fatty acids contained in the colostrum and milk of sows of native breeds on piglet rearing. *Anim. Sci. J.*, 86(1): 83–91.
- Theil, P.K., Flummer, C., Hurley, W.L., Kristensen, N.B., Labouriau, R.L. and Sørensen, M.T. (2014) Mechanistic model to predict colostrum intake based on deuterium oxide dilution technique data and impact of gestation and prefarrowing diets on piglet intake and sow yield of colostrum. *J. Anim. Sci.*, 92(12): 5507–5519.
- Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Pena, A.G., Goodrich, J.K. and Gordon, J.I. (2010). QIIME allows analysis of high-throughput community sequencing data. *Nat. Methods*, 7(5): 335-336.
- Heo, J.M., Opapeju, F.O., Pluske, J.R., Kim, J.C., Hampson, D.J. and Nyachoti, C.M. (2012) Gastrointestinal health and function in weaned pigs: A review of feeding strategies to control post-weaning diarrhoea without using in-feed antimicrobial compounds. *J. Anim. Physiol. Anim. Nutr.* (Berl), 97(2): 207–237.
- Pupa, P., Apiwatsiri, P., Sirichokchatchawan, W., Pirarat, N., Maison, T., Koontanatechanon, A. and Prapasarakul, N. (2021) Use of *Lactobacillus plantarum* (strains 22F and 25F) and *Pediococcus acidilactici* (strain 72N) as replacements for antibiotic-growth promotants in pigs. *Sci. Rep.*, 11(1): 12028.
- Wang, S., Yao, B., Gao, H., Zang, J., Tao, S., Zhang, S., Huang, S., He, B. and Wang, J. (2019) Combined supplementation of *Lactobacillus fermentum* and *Pediococcus acidilactici* promoted growth performance, alleviated inflammation, and modulated intestinal microbiota in weaned pigs. *BMC Vet. Res.*, 15(1): 239.
- Baker, A.A., Davis, E., Spencer, J.D., Moser, R. and Rehberger, T. (2013) The effect of a *Bacillus*-based directfed microbial supplemented to sows on the gastrointestinal microbiota of their neonatal piglets. *J. Anim. Sci.*, 91(7): 3390–3399.
- Cao, X., Tang, L., Zeng, Z., Wang, B., Zhou, Y., Wang, Q., Zou, P. and Li, W. (2020) Effects of probiotics BaSC06 on intestinal digestion and absorption, antioxidant capacity, microbiota composition, and macrophage polarization in pigs for fattening. Front. Vet. Sci., 7:570593.
- 31. Laskowska, E., Jarosz, Ł. and Grądzki, Z. (2019) Effect of multi-microbial probiotic formulation Bokashi on pro-and anti-inflammatory cytokines profile in the serum, colostrum and milk of sows, and in a culture of polymorphonuclear cells isolated from colostrum. *Probiotics Antimicrob. Proteins*, 11(1): 220–232.
- 32. Jarosz, Ł., Ciszewski, A., Marek, A., Grądzki, Z., Kaczmarek, B. and Rysiak, A. (2022) The effect of feed supplementation with EM Bokashi[®] multimicrobial probiotic preparation on selected parameters of sow colostrum and milk as indicators of the specific and nonspecific immune response. *Probiotics Antimicrob. Proteins*, 14(6): 1029–1041.
- Tsukahara, T., Inatomi, T., Otomaru, K., Amatatsu, M., Romero-Pérez, G.A. and Inoue, R. (2018) Probiotic supplementation improves reproductive performance of unvaccinated farmed sows infected with porcine epidemic diarrhea virus. *Anim. Sci. J.*, 89(8): 1144–1151.
- Devillers, N., van Milgen, J., Prunier, A. and Le Dividich, J. (2004 Estimation of colostrum intake in the neonatal pig. *Anim. Sci.*, 78(2): 305–313.
- Ren, C., Jin, J., Wang, X., Zhang, Y. and Jin, Q. (2022) Evaluation of fatty acid profile of colostrum and milk fat of different sow breeds. *Int. Dairy J.*, 126: 105250.
- 36. Settachaimongkon, S., Homyog, K., Mekboonsonglarp, W., Soonoue, P., Lerdamnuaylarp, T., Prayoonpeeraput, P. and Nuntapaitoon, M. (2023) Dynamics of fatty acid and non-volatile polar metabolite profiles in colostrum and milk depending on the lactation stage and parity number of sows.

- Sci. Rep., 13(1): 1989.
- 37. Giang, H.H., Viet, T.Q., Ogle, B. and Lindberg, J.E. (2010) Growth performance, digestibility, gut environment and health status in weaned piglets fed a diet supplemented with potentially probiotic complexes of lactic acid bacteria. *Livest. Sci.*, 129(1–3): 95–103.
- 38. Zhao, P.Y. and Kim, I.H. (2015) Effect of direct-fed microbial on growth performance, nutrient digestibility, fecal noxious gas emission, fecal microbial flora and diarrhea score in weanling pigs. *Anim. Feed Sci. Technol.*, 200: 86–92.
- Shin, D., Chang, S.Y., Bogere, P., Won, K., Choi, J.Y., Choi, Y.J., Lee, H.K., Hur, J., Park, B.Y., Kim, Y. and Heo, J. (2019) Beneficial roles of probiotics on the modulation of gut microbiota and immune response in pigs. *PLoS One*, 14(8): e0220843.
- Konstantinov, S.R., Awati, A.A., Williams, B.A., Miller, B.G., Jones, P., Stokes, C.R., Akkermans, A.D.L., Smidt, H. and de Vos, W.M. (2006) Post-natal development of the porcine

- microbiota composition and activities. *Environ. Microbiol.*, 8(7): 1191–1199.
- 41. Zhang, L., Xu, Y.Q., Liu, H.Y., Lai, T., Ma, J.L., Wang, J.F. and Zhu, Y.H. (2010) Evaluation of *Lactobacillus rhamnosus* GG using an *Escherichia coli* K88 model of piglet diarrhoea: Effects on diarrhoea incidence, faecal microflora and immune responses. *Vet. Microbiol.*, 141(1–2): 142–148.
- 42. Guo, X., Xia, X., Tang, R. and Wang, K. (2008) Real-time PCR quantification of the predominant bacterial divisions in the distal gut of Meishan and Landrace pigs. *Anaerobe*, 14(4): 224–228.
- 43. Cui, C., Shen, C.J., Jia, G. and Wang, K.N. (2013) Effect of dietary *Bacillus subtilis* on proportion of *Bacteroidetes* and *Firmicutes* in swine intestine and lipid metabolism. *Genet. Mol. Res.*, 12(2): 1766–1776.
- 44. Roongsitthichai, A. and Tummaruk, P. (2014) Importance of backfat thickness to reproductive performance in female pigs. *Thai J. Vet. Med.*, 44(2): 171–178.
