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Intestinal microbial composition changes induced by *Lactobacillus plantarum* GBL 16, 17 fermented feed and intestinal immune homeostasis regulation in pigs

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Abstract

In this study, *Rubus coreanus* (*R. coreanus*) byproducts with high polyphenol content were fermented with *R. coreanus*-derived lactic acid bacteria (*Lactobacillus plantarum* GBL 16 and 17). Then the effect of *R. coreanus*-derived lactic acid bacteria fermented feed (*RC*-LAB fermented feed) with probiotics (*Bacillus subtills*, *Aspergillus oryzae*, Yeast) as a feed additive for pigs on the composition of intestinal microbes and the regulation of intestinal immune homeostasis was investigated. Seventy-two finishing Berkshire pigs were randomly allotted to four different treatment groups and 18 replicates. *RC*-LAB fermented feed with probiotics increased the genera *Lactobacillus*, *Streptococcus*, *Mitsuokella*, *Prevotella*, *Bacteroides* spp., *Roseburia* spp., and *Faecalibacterium prausnitzii*, which are beneficial bacteria of the digestive tract of pigs. Also, *RC*-LAB fermented feed with probiotics decreased the genera *Clostridium*, *Terrisporobacter*, *Romboutsia*, *Kandleria*, *Megasphaera* and *Escherichia*, which are harmful bacteria. In particular, the relative abundance of the genera *Lactobacillus* and *Streptococcus* increased by an average of 8.51% and 4.68% in the treatment groups and the classes Clostridia and genera *Escherichia* decreased by an average of 27.05% and 2.85% in the treatment groups. In mesenteric lymph nodes (MLN) and spleens, the mRNA expression of transcription factors and cytokines in Th1 and Treg cells increased and the mRNA expression of Th2 and Th17 transcription factors and cytokines decreased, indicating a regulatory effect on intestinal immune homeostasis. RC-LAB fermented feed regulates gut immune homeostasis by influencing the composition of beneficial and detrimental microorganisms in the gut and regulating the balance of Th1/Th2 and Th17/Treg cells.

Keywords: *Rubus coreanus* byproducts, Probiotics, Pig, Intestinal microbiota, Immune homeostasis

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All experimental protocols involving animals in the present study were approved by the Institutional Animal Care and Use Committee (IACUC) of Gyeongnam National University of Science and Technology (No. 2018-5).

INTRODUCTION

The taxonomical composition of the bacterial community in the colon of a healthy adult pig is Firmicutes 35%, Bacteroidetes 21%, Proteobacteria 3%, and Spirochetes 2%. In the jejunum and ileum, 70% of the bacteria are Proteobacteria and 20% are Firmicutes. On the other hand, more than 75% are Firmicutes and about 13% are Proteobacteria in the cecum and colon. The administration of a complex lactobacilli cocktail (*Lactobacillus johnsonii* and *Lactobacillus mucosae*, isolated from healthy pig feces) reduces the number of potential entero-pathogens such as *Clostridia* and *E. coli* promoting a healthy gut in pigs [1,2].

Berry fruits such as strawberries, raspberries, and blackberries contain very high levels of polyphenols, particularly ellagitannins (cloudberry 315.1 mg/100 g of fw, red raspberry 297.3 mg/100 g of fw, strawberry 77.1 mg/100 of fw, sea buckthorn 1 mg/100 g of fw). Although ellagitannins and ellagic acid have very low bioavailability, they are metabolized as urolithins (3,4-benzocoumarin derivatives) by gut microbiota, and are circulated in plasma in the form of glucuronide and sulfate conjugates. Urolithins have anti-inflammatory, antioxidant, antimicrobial, anticarcinogenic, and antiglycative effects. Berry-derived polyphenols increase beneficial gut bacteria such as *Akkermansia muciniphila* and decrease pathogenic gut microbiota. In the clinical studies by Bouyahya et al. [3], blueberry consumption increased *Bifidobacterium* spp. (*B.* spp.) and *Lactobacillus acidophilus* (*L. acidophilus*) and decreased *Bacteroides* and *Clostridium* spp. (*C.* spp.).

One of the primary functions of the intestinal immune system is to maintain immune homeostasis between the nonpathogenic gut microbiome, harmless exogenous food antigens, pathogenic microbes, and damage-assigned self-antigens. T cells can be differentiated into $CD4^+$ T cells and $CD8^+$ T cells. Naive $CD4^+$ T cells can be differentiated into Th1, Th2, Th9, Th17, Th22, Treg cells and follicular helper T (Tfh) cells. T helper cells induce and control immune responses as part of adaptive immunity. Th1 cells induce phagocytosis and intracellular killing of microbes, mainly by releasing proinflammatory cytokine interferon (IFN)-γ, while Th2 cells induce specific responses to extracellular pathogens and cell-mediated inflammation, mainly by releasing anti-inflammatory cytokine interleukin (IL)-10 and IL-4. Th1 and Th2 cells are regulated mutually, and Th1/Th2 balance is important for removing infections. T-box expressed in T cells (T-bet) and GATA binding protein 3 (GATA3) are Th1 and Th2 polarizing transcription factors, where T-bet suppresses GATA3 transcription and function through direct protein-protein interaction, and GATA3 suppresses signal transducer and activator of transcription 4 (STAT4) expression [4].

IL-17-releasing cells, Th17 cells, show high expression of retinoid acid-related orphan receptor γt (RORγt) which is a transcriptional regulator. Cytokine IL-17 plays an essential role in neutrophil and macrophage recruitment and mediates the inflammatory response to infectious agents. Forkhead box protein P3 ($FOXP3^+$) regulatory T cells are a specialized population of $CD4^+$ T cells, which are essential for limiting immune activation and preventing systemic autoimmunity. Treg cells from the thymus derive the tolerogenic response to microbiota and food antigens in the intestine. This process occurs in gut training lymph nodes through interacting with antigen-presenting cells that convert circulating naïve T cells into Treg cells. Dysregulation of Treg cells causes many chronic inflammatory disorders, including inflammatory bowel disorder [5].

Our group investigated the effects of *R. coreanus* by-products on the gut microbiota and immune system of rats to confirm their efficacy as an animal feed additive [6]. We found that immune modulation effect by inducing T cell polarization was enhanced in the mesenteric lymph nodes (MLNs) from the rats fed with RC byproducts. In this study, fermented feed was prepared using *R. coreanus*-derived lactic acid bacteria (*L. plantarum* GBL 16, 17). The purpose of this study was to investigate the effect of combination with lactobacillus fermented feed and conventional probiotics

(*Bacillus subtills*, *Aspergillus oryzae* and Yeast) on the composition of intestinal microbes and the regulation of intestinal immune homeostasis in pigs.

MATERIALS AND METHODS

All experimental protocols involving animals in the present study were approved by the Institutional Animal Care and Use Committee (IACUC) of Gyeongnam National University of Science and Technology (No. 2018-5).

Animals and diets

The *R. coreanus* byproducts used in this experiment were made with the method of Yu et al. [6] who suggested that the remaining peel and seeds after making *R. coreanus* extracts be dried in an oven at 50℃ for 24 hours and pounded into a fine powder. *L. plantarum* GBL 16 and 17 separated from *R. coreanus* were subcultured more than two times in Man, Rogosa and Sharpe (MRS) medium (Difco, Detroit, MI, USA) to ferment the *R. coreanus* byproducts [7]. In order to make *R. coreanus*-derived LAB fermented feed (*RC*-LAB fermented feed), *R. coreanus*-derived LAB (*L. plantarum* GBL 16 and 17, 10^9 CFU/g) liquid culture was added to equal 20% of a medium composed of *R. coreanus* byproducts 20%, molasses 13%, corn 15%, and soybean meal 32%, and then solid fermented (25 ± 3℃ for 4 to 5 days), dried (less than 12% moisture content), and powdered to a size of 60 mesh. *RC*-LAB fermented feed (*L. plantarum* 10^6 CFU/g) and Bacillus subtills (10^8 CFU/g, EUNJIN BIO, Cheonan, Korea) 0.2%, Aspergillus Oryzae (10^7 CFU/g, EUNJIN BIO) 0.2%, Yeast (10^8 CFU/g, EUNJIN BIO) 0.2% was mixed to make a fermented probiotic feed.

Data were collected from July to 2018 to September 2018 in the Dasan Genetics farm (Namwon, Korea), which has the temperature-controlled close facilities through a ventilation system. Artificial light was provided from 8:00 to 18:00. Seventy-two finishing Berkshire pigs were randomly allotted (at approximately 65 kg body weight) to four different treatment groups (A: barrows fed with the basal diet; B: barrows fed with 0.3% *RC*-LAB fermented feed + probiotics; C: gilts fed with the basal diet; and D: gilts fed with 0.3% *RC*-LAB fermented feed + probiotics). The animals were fed *ad libitum* the assigned diet and water and tested for 75 days with eighteen replicates per treatment group. Body weights were measured twice at the beginning and end of the experiment. The dietary treatment was either a commercial finishing pig diet based on corn and soybean meal (control groups; A and C) or the diet supplemented with 0.3% *RC*-LAB fermented feed + probiotics (treatment groups; B and D). The corn-soybean meal-based commercial pig diet contained 3,306 kcal of metabolizable energy/kg, 0.88% standardized ileal digestibility of lysine, 0.4% total calcium, and 0.28% digestible phosphorus. Both dietary treatments were formulated to meet or exceed the requirements of the National Research Council [8] of finishing pigs and to have comparable metabolizable energy, crude protein (CP), standardized ileal digestibility of lysine, total calcium, and digestible phosphorus levels (Table 1).

DNA extraction and next-generation sequencing (Illumina MiSeq) from ileal contents

Ileal contents were collected for each group after harvest to extract the genomic DNA of the intestinal microorganisms in the pigs. Genomic DNA from the ileal content samples was extracted using Fecal DNA MiniPrepKits™ (Zymo Research, Irvine, CA, USA) and the sequence was analyzed via next-generation sequencing using the Illumina Miseq platform (Illumina, San Diego, CA) and CLcommunity™ software [9].

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Table 1. Composition of experimental diet for finishing pigs (as-fed basis)[1)]

Item	Finishing phase
Ingredients (%)	100.00
Corn	66.18
Soy bean meal	16.58
Mixed animal fat	5.27
DDGS	5.00
Molasses	2.50
Rice bran	2.00
Limestone	0.58
Monocalcium phosphate	0.50
L-Lysine	0.50
Salt	0.40
L-Threonine	0.12
Mineral premix[2)]	0.10
Vitamin premix[3)]	0.10
DL-Methionine	0.09
Phytase	0.03
Tryptophan	0.05
Calculated energy (Mcal/kg) and nutrient contents (%)	
Metabolizable energy	3.33
Crude protein	14.50
Crude fat	8.66
Crude fiber	2.52
Calcium	0.40
Phosphorus	0.47
Lysine	0.98
Methionine	0.31
Threonine	0.64
Tryptophan	0.17

[1)]Diets were fed *ad libitum* during the whole experimental period

[2)]Provided the following quantities of minerals per kg of complete diet: Se, 0.1 mg; I, 0.3 mg; Mn, 24.8 mg; $Cu{\cdot}SO_4$, 54.1 mg; Fe, 127.3 mg; Zn, 84.7 mg; Co, 0.3 mg.

[3)]Provided the following quantities of vitamins per kg of complete diet: vitamin A, 8,000 IU; vitamin D_3, 1,600 IU; vitamin E, 32 IU; d-biotin, 64 g; riboflavin, 3.2 mg; calcium pantothenic acid, 8 mg; niacin, 16 mg; vitamin B_{12}, 12 g; vitamin K, 2.4 mg.

DDGs, dried distiller's grains with solubles.

Quantitative polymerase chain reaction and real-time reverse transcriptase polymerase chain reaction

qPCR was then used to analyze the gut microbes containing gut short-chain fatty acids (SCFAs)-producing bacteria with the extracted DNA using a Rotor-Gene SYBR® Green PCR Kit (Qiagen, Hilden, Germany) and Rotor-Gene Q (Qiagen) [6]. Primers of 12 types of intestinal microbes are shown in Table 2. Pigs were sacrificed to analyze the intestinal immune homeostasis regulation effects of the *RC*-LAB fermented feed with probiotics. MLNs and spleen were separated from the pigs, and a TRIzol® (Life Technologies, Paisley, UK) reagent was added to them. Then it was homogenized with SilentCrusher M (Heiodlph, Germany). RNA was separated and cDNA was synthesized according to the manufacturer's instructions using a PrimeScript™ One Step RT-PCR Kit Ver.2 (TAKARA BIO, Otsu, Tokyo). RT-PCR was performed using a GeneAMP®

Table 2. Quantitative real-time polymerase chain reaction primer design

Target group/specificity	Primer sequence	
Universal	Forward	GTGSTGCAYGGYYGTCGTCA
	Reverse	ACGTCRTCCMCNCCTTCCTC
Bacteroides spp.	Forward	GAAGGTCCCCCACATTG
	Reverse	CGCKACTTGGCTGGTTCAG
Roseburia spp. and *E. rectale*	Forward	GCGGTRCGGCAAGTCTGA
	Reverse	CCTCCGACACTCTAGTMCGAC
Faecalibacterium prausnitzii	Forward	GGAGGAAGAAGGTCTTCGG
	Reverse	AATTCCGCCTACCTCTGCACT
Cluster IV *Ruminococcus* spp.	Forward	GGCGGCYTRCTGGGCTTT
	Reverse	CCAGGTGGATWACTTATTGTGTTAA
Bifidobacterium spp.	Forward	TCGCGTCYGGTGTGAAAG
	Reverse	GGTGTTCTTCCCGATATCTACA
Methanogens	Forward	GGATTAGATACCCSGGTAGT
	Reverse	GTTGARTCCAATTAAACCGCA
Oscillospira spp.	Forward	ACGGTACCCCTTGAATAAGCC
	Reverse	TCCCCGCACACCTAGTATTG
Leuconostoc mesenteroid	Forward	TGATGCATAGCCGAGTTGAG
	Reverse	GAAAGCCTTCATCACACACG
Leuconostoc citreum	Forward	GGAAACAGATGCTAATACCGAATA
	Reverse	TTTACCCCACCAACTAACTAATG
Weissella cibaria	Forward	GGGAAACCTACCTCTTAGCA
	Reverse	GGACCATCTCTTAGTGATAGCA
Weissella koreensis	Forward	GGGCTACACACGTGCTACAA
	Reverse	GATTCCGACTTCGTGTAGGC
Lactobacillus sakei	Forward	CCATGTGTAGCGGTGAAATG
	Reverse	ATCCTGTTTGCTACCCATGC

Adapted from Da Yoon Yu et al. with CC-BY-NC [6].

PCR System 9700 (Thermo Fisher Scientific, Waltham, MA, USA). The mRNA expression of transcription factors and cytokines was used to analyze T-cell polarization. The separation and analysis of RNA from the spleens and MLNs and the cycling conditions for all primers, qPCR, and RT-PCR were done according to the methods reported by Yu et al. [6]. Relative mRNA expression was normalized to glycolytic glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression levels. T1, T2, T3 and Treg-cells transcription factors and cytokines primers are shown in Table 3.

Statistical analysis

All results were represented by mean±standard deviation and SPSS Statistics v20 (IBM, Armonk, NY, USA) was used to analyze Duncan's multiple range test at $p < 0.05$.

RESULTS AND DISCUSSION

Body weight gain in pigs

The effect of feeding 0.3% *RC*-LAB fermented feed with probiotics on body weight gain is shown in Table 4. The results show that there was no effect on treatment group body weight gain, especially between groups A and B, and between C and D. Bunte et al. [10] and Min et al. [11] reported that

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Table 3. Reverse transcriptase polymerase chain reaction primer design

Target group/specificity	Primer sequence	
TGF-β	Forward	GAAGGCAGAGTTCAGGGTCTT
	Reverse	GGTTCCTGTCTTTGTGGTGAA
IL-10	Forward	ATAACTGCACCCACTTCCCA
	Reverse	TCATTTCCGATAAGGCTTGG
IFN-γ	Forward	TCAAGTGGCATAGATGTGGAAGAA
	Reverse	TGGCTCTGCAGGATTTTCATG
IL-17A	Forward	TTCATCTGTGTCTCTGATGCT
	Reverse	TTGACCTTCACATTCTGGAG
IL-4	Forward	ACAGGAGAAGGGACGCCAT
	Reverse	GAAGCCCTACAGACGAGCTCA
Foxp3	Forward	CCCATCCCCAGGAGTCTTG
	Reverse	CCATGACTAGGGGCACTGTA
GATA3	Forward	CATTACCACCTATCCGCCCTATG
	Reverse	CACACACTCCCTGCCTTCTGT
T-bet	Forward	TCAACCAGCACCAGACAGAG
	Reverse	AAACATCCTGTAATGGCTTGTG
RORγt	Forward	TTCACCCCACCTCCACTG
	Reverse	TGCAAGGGATCACTTCAATTT

Adapted from Da Yoon Yu et al. with CC-BY-NC [6].

TGF-β, transforming growth factor beta; IL-10, interleukin-10; IFN-γ, interferon-γ; Foxp3, Forkhead box P3; GATA3, GATA binding protein 3; T-bet, T-box expressed in T cells; RORγt, RAR-related orphan receptor gamma T.

Table 4. Performance parameters of pigs depending on *RC*-LAB fermented feed feeding with probiotics

Performance parameters	A[1)]	B	C	D	*p*-value
Initial body weight (kg)	62.9 ± 1.1[a]	57.4 ± 2.0[a]	63.4 ± 1.7[a]	62.0 ± 1.6[a]	0.627
Finished body weight (kg)	110.3 ± 3.2[a]	105.9 ± 4.0[a]	109.7 ± 5.3[a]	106.6 ± 4.9[a]	0.733
Average daily gain (g)	717.6 ± 51.8[a]	735.4 ± 65.7[a]	633.3 ± 81.4[a]	611.2 ± 68.6[a]	0.991

Data represent means ± SD of 18 replicates.

[1)]A, barrows fed with the basal diet; B, barrows fed with 0.3% *RC*-LAB fermented feed; C, gilts fed with the basal diet; D, gilts fed with 0.3% *RC*-LAB fermented feed.

[a]Means are significantly different within the same row ($p < 0.05$).

two operational taxonomic units (OTUs) belonging to genus *Lactobacillus* and *Bifidobacterium* in the feces and digesta of the small and large intestines significantly increased, but did not affect body weight gain when liquid feed fermented with *L. plantarum, Pediococcus pentosaceus,* and *Lactococcus lactis* was fed to pigs.

Effects of *RC*-LAB fermented feed on beneficial intestinal microorganisms in pigs by next-generation sequencing, Illumina MiSeq

The effect of *RC*-LAB fermented feed and probiotics on the intestinal beneficial microbiota composition in pigs was investigated (Table 5). The intestinal microbiota composition of the pigs consisted of Firmicutes 88.58%, Proteobacteria 5.42%, Bacteroidetes 3.77%, and Actinobacteria 1.62% at the phylum level. In humans and rats, Firmicute and Bacteroidetes are dominant bacteria [12], but Firmicute and Proteobacteria were identified as prevalent bacteria in this study. Looft et al. [13] have reported that Bacteroidetes, Firmicutes, and Proteobacteria dominated porcine fecal bacterial communities at the phyla level. However, they found that Proteobacteria increased and

Table 5. Microbiota composition determined by 16S rRNA gene sequencing (Illumina MiSeq). The relative abundance of phyla, classes and genera levels of beneficial bacteria in the digestive tract of pigs

Items[2)]	Treatments, mean% (SE)[1)]			
	A	B	C	D
Firmicutes	88.58 (2.83)[a]	85.24 (1.26)[a]	88.75 (3.82)[a]	86.42 (4.34)[a]
Bacilli	13.46 (0.85)[a]	24.09 (2.60)[b]	11.14 (0.80)[a]	24.12 (3.19)[b]
Lactobacillus	8.91 (0.74)[a]	16.83 (1.27)[b]	9.69 (0.50)[a]	18.79 (1.36)[b]
Streptococcus	1.75 (0.37)[a]	6.59 (0.96)[b]	1.13 (0.09)[a]	5.65 (0.65)[b]
Weissella	0.14 (0.01)[a]	0.46 (0.05)[b]	0.10 (0.07)[a]	0.49 (0.06)[b]
Pediococcus	0.01 (0.01)[a]	0.11 (0.02)[b]	0.01 (0.01)[a]	0.12 (0.01)[b]
Leuconostoc	0.01 (0.01)[a]	0.05 (0.01)[b]	0.00 (0.00)[a]	0.02 (0.01)[a]
Clostridia	65.06 (2.28)[b]	39.86 (2.19)[a]	67.62 (2.76)[b]	38.72 (1.89)[a]
Howardella	0.04 (0.01)[a]	0.25 (0.11)[b]	0.05 (0.02)[a]	0.21 (0.04)[ab]
Negativicutes	6.39 (0.83)[a]	4.34 (0.51)[a]	6.02 (1.02)[a]	4.03 (0.57)[a]
Mitsuokella	1.87 (0.30)[a]	3.10 (0.46)[b]	1.19 (0.19)[a]	2.04 (0.40)[a]
Proteobacteria	5.42 (0.55)[a]	6.13 (0.41)[a]	5.47 (0.90)[a]	6.32 (0.56)[a]
Betaproteobacteria	0.58 (0.23)[a]	1.86 (0.28)[b]	0.61 (0.06)[a]	2.30 (0.36)[b]
Parasutterella	0.30 (0.10)[a]	1.19 (0.25)[b]	0.35 (0.08)[a]	1.34 (0.21)[b]
Sutterella	0.24 (0.03)[a]	0.63 (0.12)[b]	0.26 (0.06)[a]	0.95 (0.12)[c]
Bacteroidetes	3.77 (0.44)[ab]	6.04 (0.42)[b]	2.44 (1.06)[a]	4.05 (0.86)[ab]
Bacteroidia	3.60 (0.38)[a]	3.07 (0.43)[a]	2.41 (0.34)[a]	2.88 (0.39)[a]
Prevotella	1.04 (0.26)[a]	2.10 (0.22)[b]	1.20 (0.15)[a]	2.18 (0.29)[b]
Bacteroides	0.02 (0.01)[a]	0.31 (0.04)[b]	0.02 (0.01)[a]	0.32 (0.05)[b]
Prevotellaceae_uc	0.03 (0.01)[a]	0.27 (0.05)[b]	0.08 (0.04)[a]	0.28 (0.06)[b]
Actinobacteria	1.62 (0.31)[a]	1.93 (0.69)[a]	2.30 (0.87)[a]	2.77 (1.14)[a]
Actinobacteria_c	1.54 (0.30)[a]	1.75 (0.21)[a]	2.07 (0.27)[a]	2.42 (0.31)[a]
Bifidobacterium	0.14 (0.03)[b]	0.06 (0.01)[a]	0.16 (0.03)[b]	0.07 (0.01)[a]

Data represent means ± SD of 6 replicates.

[1)]A, barrows fed with the basal diet; B, barrows fed with 0.3% *RC*-LAB fermented feed; C, gilts fed with the basal diet; D, gilts fed with 0.3% *RC*-LAB fermented feed.

[2)]Statistical tests of over-or under-representation of bacterial lineages among at each sample were made at the phylum, class and genera levels using Duncan's multiple range test.

[a–c]Means are significantly different within the same row ($p < 0.05$).

Bacteroidetes decreased when performance-enhancing antibiotics (chlortetracycline, sulfamethazine, and penicillin [known as ASP250]) were fed to pigs. Obese mice have fewer Bacteroidetes and more Firmicutes in their feces compared to lean mice. This shift of the fecal bacterial community is related to the higher energy-harvesting capacity of the obese mice. Also, a higher growth rate in pigs is related to a higher feed conversion rate [13].

The relative abundance of phyla Firmicutes, which was the most dominant phyla in the pigs' intestines of this study, was not different among the treatment groups (A, B, C, D), but that of class Bacilli was 12.05% higher in the treatment groups (B, D; 24.55% on average) than in the control groups (A, C; 12.5% on average) ($p < 0.05$). Genera *Lactobacillus, Streptococcus, Weissella, Pediococcus, Leuconostoc*, which belong to class Bacilli, were all significantly higher in the treatment groups (B, D) than in the control groups (A, C) ($p < 0.05$). In particular, genera *Lactobacillus* and *Streptococcus* increased by 8.51% and 4.68% on average in the treatment groups B and D, respectively. The relative abundance of class Clostridia, which accounts for most of phyla Firmicutes, significantly decreased in the treatment groups (B, D) compared to the control groups (A, C) ($p < 0.05$), but genera *Howardella* significantly increased in the control group (A) within the barrow groups (A,

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C) ($p < 0.05$). The relative abundance of class Negativicutes was not significantly different between the control groups (A, C) and the treatment groups (B, D), but genera *Mitsuokella* was significantly higher in the barrow treatment group (B) compared to the barrow control group (A) ($p < 0.05$).

Important probiotic microorganisms in genus *Lactobacillus* include LAB species (*L. acidophilus, Lacticaseibacillus casei, L. gasseri, Limosilactobacillus reuteri,* and *L. helveticus*) and *Enterococcus faecalis*, *Enterococcus faecium, Lactococcus lactis, Leuconostoc mesenteroides, Pediococcus acidilactici, Sporolactobacillus inulinus*, and *Streptococcus thermophilus* species. Probiotics have the ability to maintain a proper balance between pathogens and beneficial bacteria to prevent gastrointestinal infections and disorders, and also have an immunomodulatory action on the host, which helps remove cholesterol.

Lactic acid (LA), one of the LAB metabolites, impairs the pH gradient between cytosol (alkaline) and the external environment (acidic), and dissipates membrane potential to achieve antimicrobial activity through the destruction of pathogenic cells. Bacteriocins are heat- and acid-resisting LAB oligopeptides, which have antimicrobial activity against pathogenic bacteria and fungi [14]. *Clostridium* clusters XIVa and IV are predominant intestinal bacteria, accounting for 10% to 40% of the total bacteria, and are well known as indispensable regulators of internal homeostasis [15].

As weaned pigs age, the relative abundances of 7 bacterial genera (*Fibrobacter, Collinsella, Roseburia, Prevotella, Dorea, Howardella, and Blautia*), including *Howardella*, significantly increase. The functional maturation of this gut bacterial community is associated with the digestive system, glycan biosynthesis and metabolism, and vitamin B biosynthesis [16]. When comparing low-birthweight (LBW) piglets to normal-birth-weight piglets, LBW piglets have lower rates of *Faecalibacterium* at three days of age, *Flavonifractor* at seven days of age, *Lactobacillus, Streptococcus,* and *Prevotella* at twenty-one days of age, and *Howardella* at twenty-one and thirty-five days of age. Metabolomics analysis suggests that these lower rates are related to fatty acid metabolism, amino acid metabolism, as well as bile acid biosynthesis [17]. *L. reuteri* ZLR003 affects the fecal microbiota composition of piglets, which is associated with the metabolism of SCFAs, long chain fatty acids, and free amino acids (FAAs). In particular, genera *Mitsuokella* and *Megasphaera* have significant positive effects on serum FAAs content [18].

The relative abundance of phyla Proteobacteria, the second most dominant phyla in porcine intestines, was not different between the control and the treatment groups (A, B, C, D), but classes Betaproteobacteria, genera *Parasutterella* and *Sutterella* were significantly higher in the treatment groups (B, D) compared to the control groups (A, C) ($p < 0.05$). Genera *Parasutterella* sp. (*Parasutterella excrementihominis* species, family Sutterellaceae, order Burkholderiales, class Betaproteobacteria, and phylum Proteobacteria) is a core component of human and animal gut microbiota and known to be asaccharolytic, a succinate producer, and a high L-cysteine consumer. In response to the carbohydrate-rich diet, genera *Parasutterella* sp. activates the fatty acid biosynthesis pathway, resulting in increased body weight gain and obesity development [19]. Dietary fat intake (total fatty acids, saturated fatty acids, trans fatty acids, monounsaturated fatty acids, polyunsaturated fatty acids, n3-FAs, and n6-FAs) affects the bacterial composition and structure, and *Sutterella, Fusobacterium*, and *Tyzzerell* increase with fatty acid intake.

Phyla Bacteroidetes and classes Bacteroidia, which is the third dominant group of the porcine intestines in this study, showed no difference between the control and the treatment groups (A, B, C, D). However, genera *Prevotella, Bacteroides, Prevotellaceae_uc* significantly increased in the treatment groups (B, D) compared to the control groups (A, C) ($p < 0.05$). The *Prevotella* abundance in the pig gastrointestinal tract (GIT) varies depending on the growth stage. It is less abundant in the suckling and nursery stages and is more dominant in the growing and finishing stages after weaning. After post-weaning, *Prevotella*-enriched gut microbiota can improve the

growth performance of pigs because it increases energy harvested by fermenting complex dietary polysaccharides [20].

For phyla Actinobacteria and classes Actinobacteria_c, there was no difference between the control and the treatment groups (A, B, C, D). However, genera Bifidobacterium significantly decreased in the treatment groups (B, D) compared to the control groups (A, C) ($p < 0.05$). Actinobacteria are gram-positive bacteria with high guanine and cytosine (GC) DNA content, and they belong to one of the largest bacteriological phyla. Actinobacteria have an extensive secondary metabolism and produce about two-thirds of all naturally derived antibiotics used in clinics [21]. *Bifidobacteria* and *Lactobacilli* are dominant members of the gut microbiota of suckling pigs and are a probiotic bacterial species with various immunomodulatory properties [22].

Effects of *RC*-LAB fermented feed with probiotics on harmful intestinal microorganisms by next-generation sequencing, Illumina MiSeq

The effect of *RC*-LAB fermented feed with probiotics on the intestinal microbiota composition was analyzed for harmful microorganisms. There was no difference between the control and the treatment groups (A, B, C, D) in the relative abundance of phyla Firmicutes (Table 6). There also was no difference between barrows (A) and gilts (C) control groups in the relative abundance of

Table 6. Microbiota composition determined by 16S rRNA gene sequencing (Illumina MiSeq). The relative abundance of phyla, classes and genera levels of harmful bacteria in the digestive tract of pigs

Items[2]	Treatments, mean% (SE)[1]			
	A	B	C	D
Firmicutes	88.58 (2.83)^{a}	85.24 (1.26)^{a}	88.75 (3.82)^{a}	86.42 (4.34)^{a}
Clostridia	65.06 (2.28)^{b}	39.86 (2.19)^{a}	67.62 (2.76)^{b}	38.72 (1.89)^{a}
Clostridium	20.59 (3.17)^{b}	6.28 (0.95)^{a}	19.64 (2.81)^{b}	5.26 (1.47)^{a}
Terrisporobacter	19.69 (3.68)^{b}	7.86 (1.93)^{a}	27.83 (2.19)^{c}	6.06 (1.29)^{a}
Romboutsia	11.84 (2.41)^{bc}	5.48 (1.38)^{a}	12.90 (2.56)^{c}	6.65 (1.20)^{ab}
Asaccharospora	0.10 (0.03)^{bc}	0.03 (0.01)^{a}	0.12 (0.03)^{c}	0.04 (0.01)^{ab}
Erysipelotrichi	3.16 (0.38)^{a}	3.18 (0.40)^{a}	3.47 (0.24)^{a}	9.30 (2.86)^{b}
Kandleria	1.31 (0.42)^{ab}	0.29 (0.07)^{a}	1.78 (0.48)^{b}	0.48 (0.44)^{a}
Sharpea	0.47 (0.09)^{c}	0.25 (0.05)^{ab}	0.39 (0.05)^{bc}	0.14 (0.04)^{a}
Negativicutes	6.39 (0.83)^{a}	4.34 (0.51)^{a}	6.02 (1.02)^{a}	4.03 (0.57)^{a}
Megasphaera	4.46 (0.61)^{b}	1.57 (0.27)^{a}	3.66 (0.61)^{b}	1.59 (0.26)^{a}
Dialister	0.56 (0.09)^{b}	0.11 (0.03)^{a}	0.46 (0.06)^{b}	0.16 (0.05)^{a}
Proteobacteria	5.42 (0.55)^{a}	6.13 (0.41)^{a}	5.47 (0.90)^{a}	6.32 (0.56)^{a}
Epsilonproteobacteria	0.95 (0.22)^{b}	0.22 (0.05)^{a}	0.88 (0.06)^{b}	0.23 (0.13)^{a}
Campylobacter	0.45 (0.08)^{b}	0.02 (0.01)^{a}	0.74 (0.17)^{b}	0.08 (0.04)^{a}
Helicobacter	0.50 (0.22)^{b}	0.15 (0.09)^{a}	0.14 (0.05)^{a}	0.01 (0.00)^{a}
Gammaproteobacteria	3.87 (0.58)^{b}	0.92 (0.11)^{a}	3.86 (0.67)^{b}	1.19 (0.20)^{a}
Escherichia	3.85 (0.74)^{b}	0.82 (0.18)^{a}	3.84 (0.69)^{b}	1.17 (0.15)^{a}
Actinobacteria	1.62 (0.31)^{a}	1.93 (0.69)^{a}	2.30 (0.87)^{a}	2.77 (1.14)^{a}
Actinobacteria_c	1.54 (0.30)^{a}	1.75 (0.21)^{a}	2.07 (0.27)^{a}	2.42 (0.31)^{a}
Corynebacterium	0.14 (0.03)^{b}	0.06 (0.01)^{a}	0.16 (0.03)^{b}	0.07 (0.01)^{a}

Data represent means ± SD of 6 replicates.

[1]A, barrows fed with the basal diet; B, barrows fed with 0.3% *RC*-LAB fermented feed; C, gilts fed with the basal diet; D, gilts fed with 0.3% *RC*-LAB fermented feed.

[2]Statistical tests of over-or under-representation of bacterial lineages among at each sample were made at the phylum, class and genera levels using Duncan's multiple range test.

$^{a-c}$Means are significantly different within the same row ($p < 0.05$).

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class Clostridia, which accounts for most of the phyla Firmicutes. However, the relative abundance of class Clostridia in the controls (A, C) and the treatment groups (B, D) was on average 66.34% and 39.29%, respectively, meaning there was a significant decrease of 27.05% in the treatment groups (B, D) ($p < 0.05$). *Clostridium, Terrisporobacter, Romboutsia,* and *Asaccharospora* at a genus level significantly decreased in the treatment groups (B, D) compared to the control groups (A, C) ($p < 0.05$). In particular, the relative abundance of *Clostridium, Terrisporobacter*, and *Romboutsia* decreased by 14.41%, 16.88%, and 6.31% on average in the treatment groups (B, D). Genus *Terrisporobacter* was significantly lower in the gilts control group (C) compared to the barrows control group (A), which means there is a significant difference between sexes ($p < 0.05$). *Clostridium* spp. has about 200 distinct species, in which *Clostridium* is a heterogeneous group with pathogenic and probiotic characteristics. *Terrisporobacte*r is an emerging anaerobic pathogen, and *Romboutsia* and *Asaccharospora* are closely related to genus *Clostridium*.

The relative abundance of class Erysipelotrichi was significantly higher in the gilts treatment group (D) compared to the gilts control group (C) ($p < 0.05$). However, genus *Kandleria* was significantly lower in the gilts treatment group (D) compared to the gilts control group (C) ($p < 0.05$), and genus *Sharpea* was significantly lower in both the barrows and the gilts treatment groups (B, D) ($p < 0.05$). Although the relative abundance of class Negativicutes was no different between the control and the treatment groups, genus *Megasphaera* and *Dialiste* were significantly lower in the treatment groups (B, D) ($p < 0.05$).

The relative abundance of phyla Proteobacteria was not different between the control and the treatment groups, but class Epsilonproteobacteria was significantly lower in both treatment groups (B, D) ($p < 0.05$). Genus *Campylobacter* was also significantly reduced in the treatment groups (B, D) ($p < 0.05$). Genus *Helicobacter* was significantly reduced in the barrows treatment group (B) ($p < 0.05$). The relative abundance of class Gammaproteobacteria and genus *Escherichia* was significantly lower in both treatment groups (B, D) ($p < 0.05$). The relative abundance of genus *Campylobacter*, *Helicobacter*, and *Escherichia* in the phyla Proteobacteria was significantly lower in both treatment groups (B, D) ($p < 0.05$). In particular, *Escherichia* showed that the averages in the control groups (A, C) and the treatment groups (B, D) were 3.84% and 0.99%, respectively, which was a decrease of 2.85% on average in the treatment groups (B, D).

Presti et al. [23] confirmed Erysipelotrichi as a potential biomarker for irritable bowel syndrome (IBS), and Gammaproteobacteria, Enterococcus, and Enterococcaceae as potential biomarkers for inflammatory bowel diseases (IBD). Family Erysipelotrichaceae is abundant in the intestinal tract of mammals and is associated with host metabolic disorders, inflammatory diseases, and the concentration of N-acetylgalactosamine. Further research will be needed on the functional roles of Erysipelotrichaceae related to hosting phenotypes in the future [24]. The relative abundance of phyla Actinobacteria and class Actinobacteria_c had no difference between the control and the treatment groups, but genus *Corynebacterium* significantly decreased in the treatment groups (B, D) ($p < 0.05$). *Corynebacterium* is a diverse genus with industrial, medical or biological importance. *Corynebacterial* species are commensals, but some are notable pathogens containing the human pathogen *Corynebacterium diphtheriae* and the animal pathogen C*orynebacterium pseudotuberculosis*. *Corynebacterium ulcerans* is a zoonotic pathogen, which people often acquire from canine pets [25].

Comparison of gut short-chain fatty acids -producing bacteria *Bacteroides* sp., *Roseburia* spp., and *Faecalibacterium prausnitzii* by quantitative polymerase chain reaction

Analysis by qPCR using specific primers for twelve major types of intestinal microorganisms was performed to analyze the effect of *RC*-LAB fermented feed with probiotics on gut bacteria.

Bacteroides spp., *Roseburia* spp., and *Faecalibacterium prausnitzii* (*F. prausnitzii*) were significantly higher in the treatment groups (B, D), which were fed *RC*-LAB fermented feed ($p < 0.05$) (Fig. 1). Firmicutes, Bacteroidetes, and Proteobacteria dominated the porcine intestinal microbiota at the phylum level.

The difference in the body weight of the pigs can be explained as a difference in the intestinal microbiota. For example, pigs with better feed efficiency tend to have a higher total volatile fatty acids concentration in the cecum and a higher butyric acid concentration in the colon, which can be explained as a difference in microbial composition and function.

Bacteroidetes spp., *Roseburia* species (belonging to the phylum Firmicutes, class Clostridia, order Clostridiales, and family Lachnospiraceae), and *F. prausnitzii* (phylum Firmicutes, *Clostridium* cluster IV) produce SCFAs (acetate, propionate, butyrate) using complex polysaccharides. In particular, the SCFA, butyrate, is used as an energy source for colonocytes. *F. prausnitzii* is the most abundant bacteria in healthy human and animal microbial communities [26]. It has an anti-inflammatory effect, so it is used as a potent biomarker for the diagnosis and prognosis of gut diseases [27]. Probiotics are mainly based on restoring the intestinal ecosystem balance naturally. Therefore, using the intestinal dominant commensal bacteria as next-generation probiotics (NGPs) is the most natural way to restore microbial imbalance in GIT. An alternative would be to develop fecal microbiota transplantation (FMT) strains and probiotics, parabiotics, and postbiotics for *F. prausnitzii*, one of the candidates for NGPs.

Th1, Th2, Th17, and Treg cells transcription factors and cytokines mRNA expression in Mesenteric lymph nodes and spleen for the regulation of porcine intestinal immune homeostasis

The mRNA expression of transcription factors (T-bet for Th1 cells, GATA 3 for Th2 cells, RORγT for Th17 cells, and Foxp3 for Treg cells) and cytokines (IFN-γ for Th1 cells, IL-4 for Th2 cells, IL-17 for Th17 cells, and IL-10 for Treg cells) was investigated to determine the effect of *RC*-LAB fermented feed on T cell polarization in MLN and spleens. In MLN, the mRNA expression of the transcription factors T-bet and cytokine IFN-γ in Th1 cells significantly increased in the treatment groups (B, D) compared to the control groups (A, C) ($p < 0.05$), and the mRNA expression of the transcription factors GATA-3 and cytokine IL-4 in Th2 cells significantly decreased in treatment

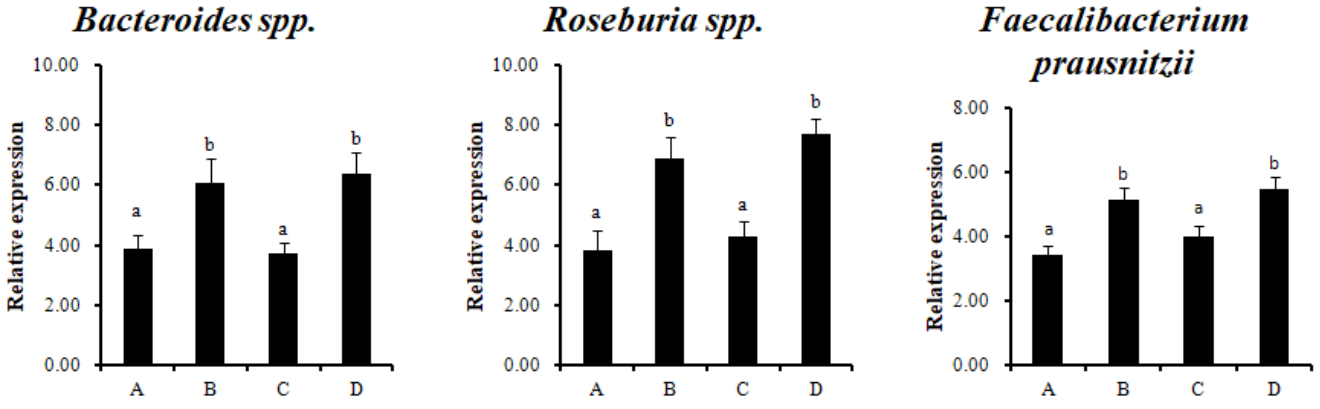

Fig. 1. Comparison of gut-dominant SCFAs-producing bacteria *Bacteroides* spp., *Roseburia* spp., and *Faecalibacterium prausnitzii* by qPCR. A, barrows fed with the basal diet; B, barrows fed with 0.3% *RC*-LAB fermented feed; C, gilts fed with the basal diet; D, gilts fed with 0.3% *RC*-LAB fermented feed. [a,b]Means are significantly different within the same row ($p < 0.05$). Data represent means ± SD of 15 replicates. SCFA, short-chain fatty acids; qPCR, quantitative polymerase chain reaction; *RC*-LAB, *R. coreanus*-derived lactic acid bacteria fermented feed.

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groups (B, D) compared to control groups (A, C) ($p < 0.05$) (Fig. 2). The mRNA expression of the transcription factors RORγ-T and cytokine IL-17 in Th17 cells significantly decreased in the treatment groups (B, D) compared to the control groups (A, C) ($p < 0.05$), and the mRNA expression of the transcription factors Foxp3 and cytokine IL-10 in Treg cells significantly increased in the treatment groups (B, D) compared to the control groups (A, C) ($p < 0.05$).

In the spleens, the mRNA expression of cytokine IFN-γ in Th1 cells increased significantly in the barrows treatment group (B), and the mRNA expression of transcription factor T-bet increased significantly in the gilts treatment group (D) ($p < 0.05$) (Fig. 3). The mRNA expression of transcription factors GATA-3 and cytokine IL-4 in Th2 cells significantly decreased in the barrows treatment group (B) ($p < 0.05$), and there was no significant difference in the gilts. In addition, the mRNA expression of the transcription factors RORγ-T and cytokine IL-17 in Th17 cells significantly decreased in the barrows treatment group (B) ($p < 0.05$), and cytokine IL-17 mRNA expression significantly decreased in the gilts ($p < 0.05$). For the mRNA expression of transcription factors Foxp3 and cytokine IL-10 in Treg cells, cytokine IL-10 significantly increased in the barrows treatment group (B) ($p < 0.05$). In the gilts treatment group (D), the mRNA expression of transcription factors Foxp3 and cytokine IL-10 significantly increased ($p < 0.05$). Feeding the *RC*-LAB fermented diet increased the transcription factors and cytokine transcription levels of Th1 cells and Treg cells in MLN, while decreasing the transcription levels of cytokines and the transcription factors of Th2 and Th17 cells. However, the immune modulatory function of Th1/Th2 and Th17/Treg in the spleens was less pronounced than in MLN.

In atopic dermatitis (AD)-induced mice treated with Duolac adenosine triphospha (ATP) (*L. casei*, *L. plantarum*, *L. rhamnosus*, and *B. lactis*), the Th1-mediated response upregulated downstream signaling molecules T-bet, STAT-1, and STAT-4 as well as IL-2 and IFN-gamma. Conversely,

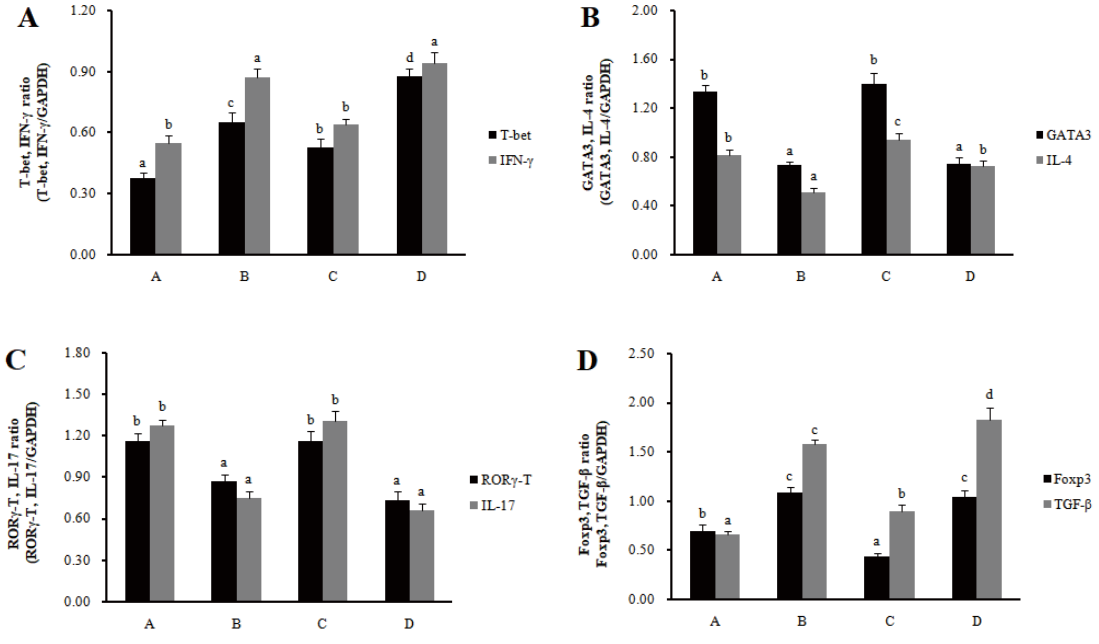

Fig. 2. Th1, Th2, Th17 and Treg cells transcription factors and cytokines mRNA expression in MLN for the regulation of porcine intestinal immune homeostasis. A, barrows fed with the basal diet; B, barrows fed with 0.3% *RC*-LAB fermented feed; C, gilts fed with the basal diet; D, gilts fed with 0.3% *RC*-LAB fermented feed. [a-c]Means are significantly different within the same row ($p < 0.05$). Data represent means±SE of 15 replicates. T-bet, T-box expressed in T cells; IFN, interferon; GATA3, GATA binding protein 3; IL, interleukin; RORγt, retinoid acid-related orphan receptor γt; FOXP3+, forkhead box protein P3; TGF, transforming growth factor; MLN, mesenteric lymph nodes; *RC*-LAB, R. coreanus-derived lactic acid bacteria fermented feed.

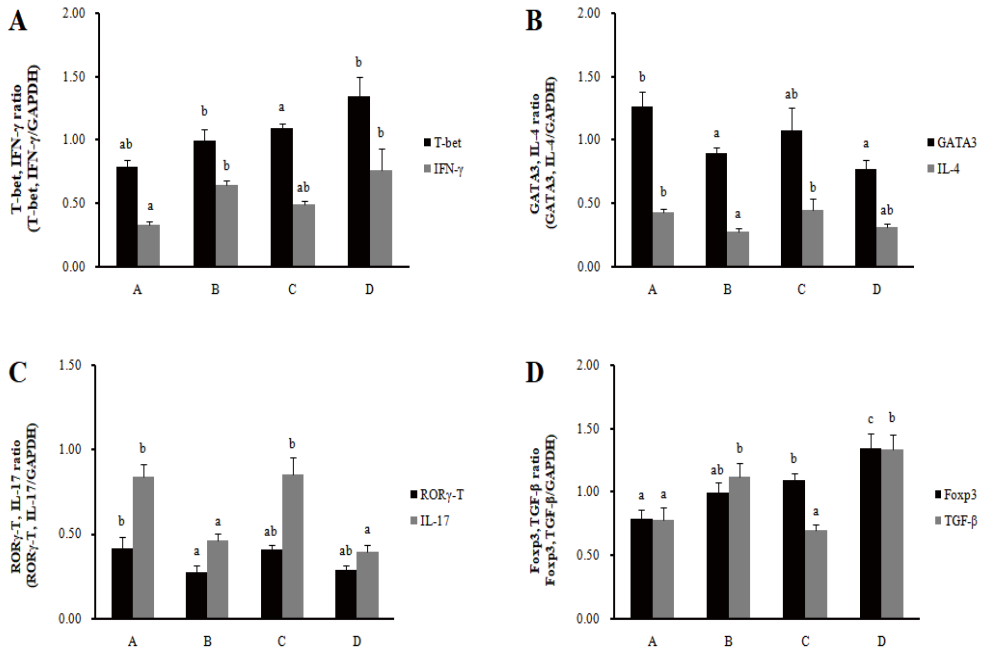

Fig. 3. Th1, Th2, Th17 and Treg cells transcription factors and cytokines mRNA expression in spleen for the regulation of porcine intestinal immune homeostasis. A, barrows fed with the basal diet; B, barrows fed with 0.3% *RC*-LAB fermented feed; C, gilts fed with the basal diet; D, gilts fed with 0.3% *RC*-LAB fermented feed. [a–c]Means are significantly different within the same row ($p < 0.05$). Data represent means ± SE of 15 replicates. T-bet, T-box expressed in T cells; IFN, interferon; GATA3, GATA binding protein 3; IL, interleukin; RORγt, retinoid acid-related orphan receptor γt; FOXP3+, forkhead box protein P3; TGF, transforming growth factor; *RC*-LAB, *R. coreanus*-derived lactic acid bacteria fermented feed.

Duolac ATP inhibited Th2 and Th17 responses by downregulating GATA-3, C-maf, IL-4, IL-5, and IL-17. These results suggest that Duolac ATP regulates dendritic cells to initiate Th1 and Treg reactions [28]. In AD-induced mice, YK4, a probiotic mixture (*Lactobacillus acidophilus* CBT LA1, *L. plantarum* CBT LP3, *Bifidobacterium breve* CBT BR3, and *B. lactis* CBT BL3), induced the ratio of Th1 cells in spleens and the ratio of Treg cells in Peyer's patches and MLN to inhibit the Th2 cell population, and Galectin-9 partially contributed to Tregs' proliferation [29]. As a result of feeding sodium butyrate and a probiotic mixture (*B. lactis*, *L. casei*, *L. rhamnosus*, and *L. plantarum*) to AD-induced mice, allergic reactions were reduced by increasing Th1 and Treg cell differentiation in MLN and spleens [30]. In addition, in 16 month old mice, *L. rhamnosus* supplementation demonstrated the potential to increase healthy aging by mitigating the immunosenescence-associated Th1/Th2 imbalance and enhancing resistance to *E. coli* infection [31].

CONCLUSION

RC-LAB fermented feed with probiotics affected the intestinal microbiota composition of pigs, increasing beneficial and SCFAs-producing bacteria and decreasing harmful bacteria. It also modulated the balance of Th1/Th2 and Th17/Treg cells in intestinal lymph nodes and the spleen. LAB fermented feed with probiotics affected the intestinal microbiota composition of pigs and showed an effect of modulating intestinal immune homeostasis.

REFERENCES

1. Patil Y, Gooneratne R, Ju XH. Interactions between host and gut microbiota in domestic pigs:

https://doi.org/10.5187/jast.2022.e89

a review. Gut Microbes. 2020;11:310-34. https://doi.org/10.1080/19490976.2019.1690363
2. Vasquez R, Oh JK, Song JH, Kang DK. Gut microbiome-produced metabolites in pigs: a review on their biological functions and the influence of probiotics. J Anim Sci Technol. 2022;64:671-95. https://doi.org/10.5187/jast.2022.e58
3. Bouyahya A, El Omari N, El Hachlafi N, El Jemly M, Hakkour M, Balahbib A, et al. Chemical compounds of berry-derived polyphenols and their effects on gut microbiota, inflammation, and cancer. Molecules. 2022;27:3286. https://doi.org/10.3390/molecules27103286
4. Xue M, Xie J, Liu L, Huang Y, Guo F, Xu J, et al. Early and dynamic alterations of Th2/Th1 in previously immunocompetent patients with community-acquired severe sepsis: a prospective observational study. J Transl Med. 2019;17:57. https://doi.org/10.1186/s12967-019-1811-9
5. Zhang S, Gang X, Yang S, Cui M, Sun L, Li Z, et al. The alterations in and the role of the Th17/Treg balance in metabolic diseases. Front Immunol. 2021;12:678355. https://doi.org/10.3389/fimmu.2021.678355
6. Yu DY, Kim SH, Kim JA, Kim IS, Moon YS, Lee SS, et al. Effects of Rubus coreanus byproducts on intestinal microbiota and the immune modulation. Asian-Australas J Anim Sci. 2018;31:429-38. https://doi.org/10.5713/ajas.17.0733
7. De Man JC, Rogosa M, Elisabeth Sharpe M. A medium for the cultivation of lactobacilli. J Appl Bacteriol. 1960;23:130-5. https://doi.org/10.1111/j.1365-2672.1960.tb00188.x
8. NRC [National Research Council]. Nutrient requirements of swine. 11th ed. Washington, DC: National Academies Press; 2012.
9. Jeon YS, Park SC, Lim J, Chun J, Kim BS. Improved pipeline for reducing erroneous identification by 16S rRNA sequences using the Illumina MiSeq platform. J Microbiol. 2015;53:60-9. https://doi.org/10.1007/s12275-015-4601-y
10. Bunte S, Grone R, Keller B, Keller C, Galvez E, Strowig T, et al. Intestinal microbiota of fattening pigs offered non-fermented and fermented liquid feed with and without the supplementation of non-fermented coarse cereals. Microorganisms. 2020;8:638. https://doi.org/10.3390/microorganisms8050638
11. Min Y, Choi Y, Choe J, Kim Y, Jeong Y, Kim D, et al. Effects of dietary mixture of protease and probiotics on growth performance, blood constituents, and carcass characteristics of growing-finishing pigs. J Anim Sci Technol. 2019;61:272-7. https://doi.org/10.5187/jast.2019.61.5.272
12. Stojanov S, Berlec A, Štrukelj B. The influence of probiotics on the firmicutes/bacteroidetes ratio in the treatment of obesity and inflammatory bowel disease. Microorganisms. 2020;8:1715. https://doi.org/10.3390/microorganisms8111715
13. Looft T, Johnson TA, Allen HK, Bayles DO, Alt DP, Stedtfeld RD, et al. In-feed antibiotic effects on the swine intestinal microbiome. Proc Natl Acad Sci USA. 2012;109:1691-6. https://doi.org/10.1073/pnas.1120238109
14. Petrova P, Arsov A, Tsvetanova F, Parvanova-Mancheva T, Vasileva E, Tsigoriyna L, et al. The complex role of lactic acid bacteria in food detoxification. Nutrients. 2022;14:2038. https://doi.org/10.3390/nu14102038
15. Guo P, Zhang K, Ma X, He P. Clostridium species as probiotics: potentials and challenges. J Anim Sci Biotechnol. 2020;11:24. https://doi.org/10.1186/s40104-019-0402-1
16. Hu J, Nie Y, Chen J, Zhang Y, Wang Z, Fan Q, et al. Gradual changes of gut microbiota in weaned miniature piglets. Front Microbiol. 2016;7:1727. https://doi.org/10.3389/fmicb.2016.01727
17. Li N, Huang S, Jiang L, Wang W, Li T, Zuo B, et al. Differences in the gut microbiota establishment and metabolome characteristics between low- and normal-birth-weight piglets during early-life. Front Microbiol. 2018;9:1798. https://doi.org/10.3389/fmicb.2018.01798

18. Zhang D, Liu H, Wang S, Zhang W, Wang J, Tian H, et al. Fecal microbiota and its correlation with fatty acids and free amino acids metabolism in piglets after a Lactobacillus strain oral administration. Front Microbiol. 2019;10:785. https://doi.org/10.3389/fmicb.2019.00785
19. Henneke L, Schlicht K, Andreani NA, Hollstein T, Demetrowitsch T, Knappe C, et al. A dietary carbohydrate – gut Parasutterella – human fatty acid biosynthesis metabolic axis in obesity and type 2 diabetes. Gut Microbes. 2022;14:2057778. https://doi.org/10.1080/19490976.2022.2057778
20. Amat S, Lantz H, Munyaka PM, Willing BP. Prevotella in pigs: the positive and negative associations with production and health. Microorganisms. 2020;8:1584. https://doi.org/10.3390/microorganisms8101584
21. Barka EA, Vatsa P, Sanchez L, Gaveau-Vaillant N, Jacquard C, Klenk HP, et al. Taxonomy, physiology, and natural products of actinobacteria. Microbiol Mol Biol Rev. 2016;80:1-43. https://doi.org/10.1128/MMBR.00019-15
22. Mikkelsen LL, Bendixen C, Jakobsen M, Jensen BB. Enumeration of bifidobacteria in gastrointestinal samples from piglets. Appl Environ Microbiol. 2003;69:654-8. https://doi.org/10.1128/AEM.69.1.654-658.2003
23. Presti AL, Zorzi F, Chierico FD, Altomare A, Cocca S, Avola A, et al. Fecal and mucosal microbiota profiling in irritable bowel syndrome and inflammatory bowel disease. Front Microbiol. 2019;10:1655. https://doi.org/10.3389/fmicb.2019.01655
24. Wu J, Liu M, Zhou M, Wu L, Yang H, Huang L, et al. Isolation and genomic characterization of five novel strains of Erysipelotrichaceae from commercial pigs. BMC Microbiol. 2021;21:125. https://doi.org/10.1186/s12866-021-02193-3
25. Dover LG, Thompson AR, Sutcliffe IC, Sangal V. Phylogenomic reappraisal of fatty acid biosynthesis, mycolic acid biosynthesis and clinical relevance among members of the genus Corynebacterium. Front Microbiol. 2021;12:802532. https://doi.org/10.3389/fmicb.2021.802532
26. Oh JK, Vasquez R, Kim SH, Hwang IC, Song JH, Park JH, et al. Multispecies probiotics alter fecal short-chain fatty acids and lactate levels in weaned pigs by modulating gut microbiota. J Anim Sci Technol. 2021;63:1142-58. https://doi.org/10.5187/jast.2021.e94
27. Raheem A, Liang L, Zhang G, Cui S. Modulatory effects of probiotics during pathogenic infections with emphasis on immune regulation. Front Immunol. 2021;12:616713. https://doi.org/10.3389/fimmu.2021.616713
28. Kim HW, Hong R, Choi EY, Yu KS, Kim N, Hyeon JY, et al. A probiotic mixture regulates T cell balance and reduces atopic dermatitis symptoms in mice. Front Microbiol. 2018;9:2414. https://doi.org/10.3389/fmicb.2018.02414
29. Kim HW, Ju DB, Kye YC, Ju YJ, Kim CG, Lee IK, et al. Galectin-9 induced by dietary probiotic mixture regulates immune balance to reduce atopic dermatitis symptoms in mice. Front Immunol. 2020;10:3063. https://doi.org/10.3389/fimmu.2019.03063
30. Kim JA, Kim SH, Kim IS, Yu DY, Kim SC, Lee SH, et al. Anti-inflammatory effects of a mixture of lactic acid bacteria and sodium butyrate in atopic dermatitis murine model. J Med Food. 2018;21:716-25. https://doi.org/10.1089/jmf.2017.4116
31. Sharma R, Kapila R, Dass G, Kapila S. Improvement in Th1/Th2 immune homeostasis, antioxidative status and resistance to pathogenic E. coli on consumption of probiotic Lactobacillus rhamnosus fermented milk in aging mice. AGE. 2014;36:9686. https://doi.org/10.1007/s11357-014-9686-4

https://doi.org/10.5187/jast.2022.e89