Effects of Lactobacillus reuteri LR1 on Serum Biochemical Parameters and Intestinal Nutrient Transporter mRNA Expression in Weaned Piglets: Postprint

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Abstract

This experiment aimed to investigate the effects of Lactobacillus reuteri LR1 on serum biochemical indices and intestinal nutrient transporter mRNA expression in weaned piglets. A total of 144 21-day-old Duroc × Landrace × Large White weaned piglets with an initial body weight of (6.49±0.01) kg were randomly allocated into 3 groups, with 8 replicates per group and 6 piglets per replicate. The control group was fed a basal diet, the antibiotic group was fed the basal diet supplemented with 100 mg/kg olaquindox + 75 mg/kg chlortetracycline, and the Lactobacillus reuteri group was fed the basal diet supplemented with 5×10^10 CFU/kg Lactobacillus reuteri LR1. The experimental period lasted 14 days. The results showed: 1) Compared with the control group, dietary antibiotic supplementation significantly increased serum glucose (GLU) content (P<0.05) and significantly decreased serum urea nitrogen (UN) content (P<0.05). 2) Compared with the control group, dietary Lactobacillus reuteri LR1 supplementation significantly increased mRNA expression of duodenal motilin (MLN) and jejunal cholecystokinin (CCK) (P<0.05); dietary antibiotic supplementation significantly increased mRNA expression of duodenal MLN (P<0.05). 3) Compared with the control group, dietary Lactobacillus reuteri LR1 supplementation significantly increased mRNA expression of duodenal Na+-dependent glutamine carrier 2 (ASCT2), cationic amino acid transporter 1 (CAT1), peptide transporter 1 (PepT1), jejunal neutral and basic amino acid transporter (rBAT), and jejunal and ileal y+L amino acid transporter 1 (y+LAT1) (P<0.05); dietary antibiotic supplementation significantly increased mRNA expression of jejunal y+LAT1, CAT1, PepT1, and jejunal and ileal mammalian target of rapamycin (mTOR) (P<0.05). 4) Compared with the control group, dietary Lactobacillus reuteri LR1 supplementation significantly

increased mRNA expression of duodenal intestinal fatty acid-binding protein (I-FABP), acetyl-CoA carboxylase (ACC), fatty acid synthase (FASN), duodenal and jejunal fatty acid-binding protein 3 (FABP3), and duodenal, jejunal, and ileal peroxisome proliferator-activated receptor (PPAR) ($P<0.05$); dietary antibiotic supplementation significantly increased mRNA expression of jejunal ACC ($P<0.05$). 5) Compared with the control group, dietary Lactobacillus reuteri LR1 supplementation significantly increased mRNA expression of jejunal and ileal sodium-glucose cotransporter 1 (SGLT1) ($P<0.05$); dietary antibiotic supplementation significantly increased mRNA expression of duodenal SGLT1 and sodium-glucose cotransporter 3 (SGLT3) ($P<0.05$). In conclusion, dietary supplementation with 5×10^10 CFU/kg Lactobacillus reuteri LR1 exerted beneficial effects on intestinal nutrient transport in weaned piglets, as evidenced by promoting intestinal physical and chemical digestion, facilitating the absorption and transport of small peptides, amino acids, and fatty acids, and enhancing fatty acid synthesis. Lactobacillus reuteri LR1 possesses great potential as an alternative to feed antibiotics in swine and can be utilized for developing novel antibiotic substitutes in pig feed.

Full Text

Effects of *Lactobacillus reuteri* LR1 on Serum Biochemical Indexes and mRNA Expression of Intestinal Nutrient Transporters in Weaned Piglets

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Abstract

This experiment investigated the effects of *Lactobacillus reuteri* (*L. reuteri*) LR1 on serum biochemical indexes and mRNA expression of intestinal nutrient transporters in weaned piglets. A total of 144 weaned piglets (Duroc × Landrace × Yorkshire, 21 days of age) with an initial body weight of (6.49 ± 0.01) kg were randomly allocated to 3 groups with 8 replicates per group and 6 piglets per replicate. Piglets in the control group were fed a basal diet, those in the antibiotic group received the basal diet supplemented with 100 mg/kg olaquindox + 75 mg/kg aureomycin, and those in the *L. reuteri* group received the basal diet supplemented with 5×10^1 CFU/kg *L. reuteri* LR1. The experimental period lasted 14 days. The results showed: (1) Compared with the control

group, dietary antibiotic supplementation significantly increased serum glucose (GLU) content ($P < 0.05$) and significantly decreased serum urea nitrogen (UN) content ($P < 0.05$). (2) Compared with the control group, *L. reuteri* LR1 supplementation significantly increased mRNA expression of motilin (MLN) in the duodenum and cholecystokinin (CCK) in the jejunum ($P < 0.05$); antibiotic supplementation significantly increased mRNA expression of MLN in the duodenum ($P < 0.05$). (3) Compared with the control group, *L. reuteri* LR1 supplementation significantly increased mRNA expressions of Na -dependent glutamine transporter 2 (ASCT2), cationic amino acid transporter 1 (CAT1), peptide transporter 1 (PepT1) in the duodenum, neutral and basic amino acid transport protein (rBAT) in the jejunum, and y L amino acid transporter 1 (y LAT1) in both jejunum and ileum ($P < 0.05$); antibiotic supplementation significantly increased mRNA expressions of y LAT1, CAT1, PepT1 in the jejunum and mammalian target of rapamycin (mTOR) in both jejunum and ileum ($P < 0.05$). (4) Compared with the control group, *L. reuteri* LR1 supplementation significantly increased mRNA expressions of intestinal fatty acid binding protein (I-FABP), acetyl-CoA carboxylase (ACC), fatty acid synthase (FASN) in the duodenum, fatty acid binding protein 3 (FABP3) in both duodenum and jejunum, and peroxisome proliferator-activated receptor (PPAR) in duodenum, jejunum, and ileum ($P < 0.05$); antibiotic supplementation significantly increased mRNA expression of ACC in the jejunum ($P < 0.05$). (5) Compared with the control group, *L. reuteri* LR1 supplementation significantly increased mRNA expression of sodium-dependent glucose transporter 1 (SGLT1) in the jejunum and ileum ($P < 0.05$); antibiotic supplementation significantly increased mRNA expressions of SGLT1 and sodium-dependent glucose transporter 3 (SGLT3) in the duodenum ($P < 0.05$). In conclusion, dietary supplementation with 5×10^1 CFU/kg *L. reuteri* LR1 exerts favorable effects on intestinal nutrient transport in weaned piglets by promoting both physical and chemical digestion, facilitating absorption and transport of peptides, amino acids, and fatty acids, and enhancing fatty acid synthesis. *Lactobacillus reuteri* LR1 demonstrates great potential as an alternative to in-feed antibiotics for developing novel antibiotic substitutes in swine production.

Keywords: *Lactobacillus reuteri* LR1; antibiotic; weaned piglets; nutrients; transporters

Introduction

Weaning subjects piglets to environmental and nutritional stress, accompanied by profound changes in gastrointestinal physiology, microbiota, and immunity, manifested as reduced feed intake, weight loss, and decreased feed digestibility [1]. Historically, antibiotics have been used as growth promoters to overcome these problems and reduce economic losses. However, extensive antibiotic use causes negative effects such as drug residues and bacterial resistance, making antibiotic bans or restrictions imperative. Consequently, identifying antibiotic

alternatives has become an urgent task for the livestock industry. Probiotic preparations are recognized as one of the more effective antibiotic alternatives—live microorganisms that confer health benefits to the host when administered in adequate amounts [2]. Numerous studies have shown that probiotic supplementation in piglet diets improves growth performance (increasing average daily gain, average daily feed intake, and feed conversion ratio) [3], suggesting that probiotics promote nutrient absorption. On one hand, probiotics can improve apparent total tract digestibility (ATTD) of nitrogen (N) [4] and digestibility of major nutrients including crude protein, crude fat, crude fiber, crude ash, calcium, and phosphorus in piglets or growing pigs [5]. On the other hand, probiotics promote nutrient digestion and absorption by enhancing intestinal digestive enzyme activity [6]. However, the molecular mechanisms through which probiotics promote intestinal nutrient metabolism in vivo have rarely been reported. Our previous research demonstrated that *L. reuteri* LR1 improved piglet growth performance during days 1–14 post-weaning [7]. Therefore, we hypothesized that *L. reuteri* might enhance growth performance by promoting expression of intestinal nutrient transporters, thereby accelerating nutrient absorption. This study investigated the regulatory effects of *L. reuteri* LR1 on gastrointestinal hormones and transporters and key enzymes involved in nutrient metabolism to explore its influence on intestinal nutrient transport and absorption function in piglets, providing a scientific basis for the application of *L. reuteri* in swine production.

Materials and Methods

1.1 Experimental Materials The probiotic preparation used in this experiment was *L. reuteri* LR1, previously isolated, screened, and identified by the Institute of Animal Science, Guangdong Academy of Agricultural Sciences, and confirmed to meet the criteria for use as a probiotic feed additive for pigs [8]. The *L. reuteri* LR1 was spray-dried into powder for subsequent diet mixing. The antibiotics used were olaquindox and aureomycin with effective component contents of 50% and 20%, respectively.

1.2 Experimental Animals and Design This experiment employed a single-factor design. A total of 144 Duroc × Landrace × Yorkshire weaned piglets at 21 days of age [initial body weight (6.49 ± 0.01) kg] were randomly allocated to three groups: control group (CON) fed the basal diet; antibiotic group (OA) fed the basal diet + 100 mg/kg olaquindox + 75 mg/kg aureomycin; and *L. reuteri* group (LR1) fed the basal diet + 5.0×10^1 CFU/kg *L. reuteri* LR1. Each group comprised 8 replicates with 6 piglets per replicate, and the experimental period lasted 14 days.

1.3 Basal Diet and Animal Management The basal diet was formulated according to the NRC (2012) nutrient requirements for pigs in the 7–11 kg

growth phase, with composition and nutrient levels shown in Table 1 . The basal diet was prepared as powder. The experiment was conducted at the experimental farm of the Institute of Animal Science, Guangdong Academy of Agricultural Sciences. The pig house was a fully enclosed building with temperature control facilities. Piglets were housed in elevated slatted-floor pens equipped with drinkers and adjustable-flow feeders, allowing ad libitum access to feed and water. All piglets were vaccinated and managed under consistent health protocols.

Table 1 Composition and nutrient levels of the basal diet (air-dry basis)

Item	Content
Ingredients	
Corn	
Extruded corn	
Fermented soybean meal (54%)	
Extruded soybean	
Fish meal	
Whey powder	
Soybean hull	
Soybean oil	
Plasma protein powder	
White sugar	
Choline chloride (50%)	
NaCl	
CaHPO	
Limestone	
L-Lys · HCl	
DL-Met	
L-Thr	
L-Trp	
Premix[1])	
Total	
Nutrient levels[2])	
DE (MJ/kg)	
CP	
TP	
AP	
Lys	
Met + Cys	
Thr	
Trp	

[1]) The premix provided per kilogram of diet: VA 12,400 IU, VD 2,800 IU, VE 200 IU, VK 5 mg, VB 40 g, VB 3 mg, VB 10 mg, niacin 40 mg, D-

pantothenic acid 15 mg, folic acid 1 mg, VB 8 mg, biotin 0.08 mg, Fe (FeSO · H O) 120 mg, Cu (CuSO ·5H O) 16 mg, Mn (MnSO ·H O) 70 mg, Zn (ZnSO · H O) 120 mg, I (CaI O) 0.7 mg, Se (Na SeO) 0.48 mg.
[2]) Nutrient levels were calculated values except CP, Ca, and TP, which were measured values.

1.4 Sample Collection On day 14 of the experiment at 08:00, one piglet was randomly selected from each replicate in each group. Blood (5 mL) was collected from the anterior vena cava to obtain serum, which was stored at -20 °C for serum biochemical analysis. After anesthesia and exsanguination, the abdominal cavity was opened to completely remove the digestive tract and separate internal organs. The small intestine was anatomically divided into duodenum, jejunum, and ileum using sutures. Approximately 2 cm of the middle segment from each intestinal section was collected, rinsed with ice-cold phosphate-buffered saline (PBS) to remove contents, blotted dry with filter paper, placed in sterile EP tubes, snap-frozen in liquid nitrogen, and subsequently stored at -80 °C.

1.5 Laboratory Analyses

1.5.1 Serum Biochemical Indexes Serum contents of albumin (ALB), globulin (GLB), total protein (TP), urea nitrogen (UN), and glucose (GLU), as well as activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH) were determined using commercial kits according to manufacturer protocols.

1.5.2 Real-Time Fluorescent Quantitative PCR for Gastrointestinal Hormones and Intestinal Nutrient Transporter mRNA Expression

1.5.2.1 Total RNA Extraction and cDNA Synthesis

Frozen intestinal tissue samples were ground into powder in a pre-sterilized mortar with liquid nitrogen. Appropriate amounts of powder were transferred to sterile EP tubes, and total RNA was extracted using the Trizol method. Total RNA was reverse-transcribed into cDNA using a kit (TaKaRa, Japan), and the cDNA was diluted to appropriate concentrations for quantitative real-time PCR (qPCR).

1.5.2.2 qPCR Primer Design

Target gene sequences were obtained from NCBI, and primers were designed using Prime Primer 5 software. Primers were synthesized by Shanghai Sangon Biotech Co., Ltd., with sequences and parameters shown in Table 2 .

Table 2 Primers parameters for qPCR

Genes	Accession No.	Primer sequences (5’ -3’)	Product size/bp
I-FABP	NM_001031780.1	F: AGGAGGCAGAA-GAAAACAATCACR: AATCACTTTAATAG-CATAGGGACA	
FABP3	NM_001099931.1	F: ACGGGGAACTTCA-GAGGATGR: GATTCCAATGTC-CACAGGCG	
ACC	NM_001114269.1	F: TGGTGCCTGGAA-GATAGACCR: TCCCAGT-GAGTTCAGTTCCG	
FASN	NM_001099930.1	F: CTGGGAGTG-GAGTTTGATGAGACR: CCATGGGTGAGT-GTCAGGAT	
SREBP1	NM_214157.1	F: GGTGATGGTC-TATATCCCTCCTCR: GATTTCTACGGTCC-CTTCTGGT	
PPAR	NM_214379.1	F: TGGAGGTGCGCCA-GATACR: GGTTCAGCGTTGCCT-GCT	
ASCT2	XM_003127238.5	F: GGTGCCTCTG-GTAGTGGACAR: TAGCGTTTCTCGATG-GCATT	
y LAT1	NM_001110421.1	F: GCTGGCCTC-CTTGATGAATAR: CTCGAACTTGGGCTC-CATAA	
rBAT	NM_001123042.1	F: CTGGTCTCCTGGAT-CATGTGGR: CAGGAAGCG-GTAGGGGTTTT	
CAT1	XM_003127584	F: GAGTGCCAGAACA-CAAACGAR: TCCTCCATCTTC-CAAATCCA	
PepT1	NM_214347.1	F: CTACCAGGTCTACC-CTCGTTCR: TTCCCATAGACACT-CACCCA	

Genes	Accession No.	Primer sequences (5’ -3’)	Product size/bp
GLUT2	NM_001097417.1	F: CATCAAAAACTG-GCAGCTCAR: TGGTAGCGATGCAGT-CAAAG	
GLUT4	NM_001128433.1	F: AGCCCATAA-GAAAACGGGGAR: AAAGGACACCAGCC-GATGTA	
SGLT1	XM_021072101.1	F: GGTTTAGGCATCG-GAGTAAGAAGTR: GGTCAAACAAAGCCCA-GAACAT	
SGLT3	NM_014227.2	F: GCACATCCTGCTTG-GTCTATCTR: CACTTGATGCTTCTTC-CCTTTC	
CCK	NW_015386253.1	F: AGGCACCCTCACTAC-CCTCTR: CTTCTTCCTTCCCAGC-CACT	
MLN	NM_214235.1	F: TCATCATCGTCCTG-GTCGTCTCR: CTTCTGGGGCTTCTTGAAT-GTC	
mTOR	XM_003124280.4	F: ATACATCAAG-GCAGGGGTGAR: CGCCAGATAAAGGTC-CAATC	
- ACTB	NM_001206359.1	F: CACGCCATCCT-GCGTCTGGAR: AGCACCGTGTTGGCG-TAGAG	
GAPDH	XM_021072101.1	F: ACTCACTCTTC-CACTTTTGATGCTR: TGTTGCTGTAGC-CAAATTCA	

CCK: cholecystokinin; MLN: motilin; I-FABP: intestinal fatty acid binding protein; FABP3: fatty acid binding protein 3; ACC : acetyl-CoA carboxylase ; FASN: fatty acid synthase; SREBP1: sterol regulatory element-binding protein 1; PPAR : peroxisome proliferator-activated receptor ; ASCT2: Na -dependent glutamine transporter 2; y LAT1: y L amino acid transporter 1; rBAT: neutral and basic amino acid transport protein; GLUT: glucose transporter; SGLT:

sodium-dependent glucose transporter; CAT1: cationic amino acid transporter 1; mTOR: mammalian target of rapamycin; PepT1: peptide transporter 1; -ACTB: -actin; GAPDH: glyceraldehyde-3-phosphate dehydrogenase.

1.5.2.3 qPCR System Composition and Program Settings

The qPCR reaction system (total volume 10 L) contained 5 L iTaq Universal SYBR Green Supermix, 0.5 L each of forward and reverse primers (10 mol/L), and 4 L cDNA template. Each sample was run in duplicate. The amplification program consisted of pre-denaturation at 95.0 °C for 30 s, followed by 40 cycles of denaturation at 95.0 °C for 15 s, annealing for 30 s, and extension at 72.0 °C for 30 s, followed by melt curve analysis.

1.6 Data Processing Data were analyzed using the one-way ANOVA module of SPSS 18.0 software. Multiple comparisons were performed using the least significant difference (LSD) method with significance level set at $P < 0.05$. Results are expressed as mean ± standard error (mean ± SE).

Results

2.1 Effects of *L. reuteri* LR1 on Serum Biochemical Indexes of Weaned Piglets As shown in Table 3 , on day 14 of the experiment, dietary antibiotic supplementation significantly increased serum glucose content and significantly decreased serum urea nitrogen content compared with the control group ($P < 0.05$), with no significant effects on other serum biochemical indexes ($P > 0.05$). Dietary *L. reuteri* LR1 supplementation showed no significant effects on serum biochemical indexes ($P > 0.05$) but exhibited a tendency to decrease urea nitrogen content by 29.11% ($P > 0.05$).

Table 3 Effects of *L. reuteri* LR1 on serum biochemical indexes of weaned piglets

Items	Control	Antibiotic	*L. reuteri* LR1
Albumin ALB (g/L)	28.64 ± 1.07	28.74 ± 1.20	28.45 ± 1.24
Globulin GLB (g/L)	24.06 ± 1.45	23.7 ± 2.08	21.33 ± 1.70
Total protein TP (g/L)	52.70 ± 1.50	52.44 ± 2.45	49.78 ± 1.42
AST (U/L)	94.63 ± 9.09	100.50 ± 14.72	91.38 ± 7.39
ALT (U/L)	56.50 ± 7.11	74.63 ± 12.66	61.00 ± 5.41
ALP (U/L)	373.00 ± 44.90	399.88 ± 45.43	358.62 ± 37.68
UN (mmol/L)	3.95 ± 0.42	2.73 ± 0.24	2.51 ± 0.48
LDH (U/L)	1,164 ± 89	1,153 ± 143	1,017 ± 129
GLU (mmol/L)	4.13 ± 0.52	2.80 ± 0.45	3.04 ± 0.26

In the same row, values with no letter or the same letter superscripts indicate

no significant difference (P > 0.05), while different lowercase letter superscripts indicate significant difference (P < 0.05).

2.2 Effects of *L. reuteri* LR1 on mRNA Expression of Intestinal Gastrointestinal Hormones in Weaned Piglets As shown in Figure 1 [Figure 1: see original paper], compared with the control group, dietary *L. reuteri* LR1 supplementation significantly increased mRNA expression of cholecystokinin (CCK) in the jejunum ($P < 0.05$) but significantly decreased CCK mRNA expression in the ileum ($P < 0.05$). Compared with the antibiotic group, *L. reuteri* LR1 supplementation significantly increased CCK mRNA expression in the duodenum ($P < 0.05$). Both *L. reuteri* LR1 and antibiotic supplementation significantly increased mRNA expression of motilin (MLN) in the duodenum compared with the control group ($P < 0.05$), while antibiotic supplementation also significantly decreased MLN mRNA expression in the jejunum ($P < 0.05$).

Value columns with no letters or the same letters indicate no significant difference (P > 0.05), while different lowercase letters indicate significant difference (P < 0.05). The same applies below.

Figure 1 Effects of *L. reuteri* LR1 on mRNA expressions of intestinal gastrointestinal hormones of weaned piglets

2.3 Effects of *L. reuteri* LR1 on mRNA Expression of Intestinal Nutrient Transporters and Key Enzymes in Weaned Piglets As shown in Figure 2 [Figure 2: see original paper], compared with the control group, dietary *L. reuteri* LR1 supplementation significantly increased mRNA expressions of the amino acid transporters ASCT2, CAT1, PepT1 in the duodenum, rBAT in the jejunum, and y LAT1 in both jejunum and ileum ($P < 0.05$), but significantly decreased mRNA expressions of y LAT1 in the duodenum and CAT1 in the ileum ($P < 0.05$). Antibiotic supplementation increased mRNA expressions of y LAT1, CAT1, PepT1 in the jejunum and mTOR in both jejunum and ileum ($P < 0.05$), while significantly decreasing mRNA expressions of PepT1 in the duodenum and ileum and ASCT2 in the jejunum ($P < 0.05$).

Regarding intestinal fatty acid synthesis transporters and key enzymes, Figure 3 [Figure 3: see original paper] shows that compared with the control group, *L. reuteri* LR1 supplementation significantly increased mRNA expressions of I-FABP, ACC , FASN in the duodenum, FABP3 in both duodenum and jejunum, and PPAR in duodenum, jejunum, and ileum ($P < 0.05$), but significantly decreased mRNA expressions of I-FABP in the jejunum and FABP3 and sterol regulatory element-binding protein 1 (SREBP1) in the ileum ($P < 0.05$). Antibiotic supplementation significantly increased mRNA expression of ACC in the jejunum ($P < 0.05$) but significantly decreased mRNA expressions of FABP3 and SREBP1 in the ileum ($P < 0.05$).

As shown in Figure 4 [Figure 4: see original paper], compared with the control group, *L. reuteri* LR1 supplementation significantly increased mRNA expression

of SGLT1 in the jejunum and ileum ($P < 0.05$), while antibiotic supplementation significantly increased mRNA expressions of SGLT1 and SGLT3 in the duodenum ($P < 0.05$). However, *L. reuteri* LR1 supplementation significantly decreased mRNA expression of glucose transporter 2 (GLUT2) in the jejunum ($P < 0.05$), and antibiotic supplementation significantly decreased mRNA expressions of GLUT2 in the duodenum, GLUT4 in the duodenum, and GLUT2 in the ileum ($P < 0.05$).

Figure 2 Effects of *L. reuteri* LR1 on mRNA expressions of intestinal amino acid transporters of weaned piglets

Figure 3 Effects of *L. reuteri* LR1 on mRNA expressions of intestinal transporters and key enzymes related to fatty acid synthesis of weaned piglets

Figure 4 Effects of *L. reuteri* LR1 on mRNA expressions of intestinal glucose transporters of weaned piglets

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Discussion

3.1 Effects of *L. reuteri* LR1 on Serum Biochemical Indexes of Weaned Piglets Serum biochemical indexes commonly reflect changes in organ function and metabolism. For example, serum total protein content can reflect dietary protein utilization to some extent, while globulin content indicates antibody levels [9]. Urea nitrogen, as a major product of protein metabolism, reflects protein utilization efficiency or amino acid balance status. Lower serum urea nitrogen content indicates higher nitrogen utilization efficiency, vigorous protein anabolism, weak catabolism, and favorable protein deposition [10]. In this study, dietary antibiotic supplementation significantly decreased serum urea nitrogen content, and *L. reuteri* LR1 also tended to decrease urea nitrogen content, indicating that protein anabolism exceeded catabolism in piglets, resulting in protein deposition. This partially explains the improved average daily gain observed in both the *L. reuteri* and antibiotic groups in our animal trial. Serum glucose originates primarily from intestinal absorption and hepatic glycogenolysis, with the latter being the main source under fasting conditions. The results showed that antibiotic supplementation significantly increased serum glucose content under fasting conditions, suggesting higher energy metabolism levels in this group, possibly related to significantly increased feed intake during the experimental period. Additionally, *L. reuteri* LR1 supplementation tended to increase serum glucose content, though the difference was not significant. These results are similar to those reported by Zhou et al. [9].

3.2 Effects of *L. reuteri* LR1 on Intestinal Gastrointestinal Hormones in Weaned Piglets Cholecystokinin (CCK) is a polypeptide hormone secreted by type I cells of the gastrointestinal mucosa that stimulates gallbladder contraction and promotes pancreatic digestive enzyme secretion [11]. This study

demonstrated that *L. reuteri* LR1 increased CCK mRNA expression in the jejunum, and compared with the antibiotic group, significantly increased CCK mRNA expression in the duodenum, potentially inducing greater release of bile and pancreatic digestive enzymes. This suggests that *L. reuteri* LR1 possesses stronger capacity to promote chemical digestion than antibiotics, possibly due to metabolites produced during *L. reuteri* fermentation.

Motilin (MLN) is a hormone produced by chromaffin cells in the anterior small intestine that stimulates gastrointestinal motility, particularly in the gastric antrum and anterior duodenum, and participates in interdigestive intestinal movement. Research indicates that MLN is involved in feed intake regulation and interdigestive gastrointestinal motility modulation [12]. This study showed that both *L. reuteri* LR1 and antibiotic supplementation significantly increased MLN mRNA expression in the duodenum, suggesting that both treatments can promote gastrointestinal peristalsis and segmentation, thereby enhancing physical digestion and improving intestinal nutrient absorption and utilization.

3.3 Effects of *L. reuteri* LR1 on Intestinal Amino Acid Transport in Weaned Piglets Proteins, carbohydrates, and fats are broken down into free amino acids, monosaccharides, and fatty acids in the small intestine, which are then absorbed into cells via corresponding transporters or other mechanisms for utilization [13]. Our previous animal trial indicated that *L. reuteri* LR1 improved average daily gain of weaned piglets by 22.73%, similar to the effect of antibiotics (29.63% increase), thereby improving growth performance [7]. We therefore hypothesized that *L. reuteri* LR1 might improve intestinal nutrient absorption function by promoting mRNA expression of nutrient transporters. Studies have shown that *Lactobacillus plantarum* can promote expression of amino acid transporters y LAT1 and CAT1 in porcine epithelial cells [14]. Zhou [15] found that lactobacilli significantly increased mRNA expression of b , amino acid transporter (b , AT) and y LAT1 in porcine duodenum. Our results showed that *L. reuteri* LR1 supplementation significantly increased mRNA expressions of ASCT2, CAT1, PepT1 in the duodenum, rBAT in the jejunum, and y LAT1 in both jejunum and ileum, consistent with these previous studies and similar to the effects of antibiotics, indicating that *L. reuteri* LR1 may promote amino acid absorption. However, *L. reuteri* LR1 significantly decreased mRNA expressions of y LAT1 in the duodenum and CAT1 in the ileum, possibly due to differential expression patterns of CAT1 mRNA among intestinal segments or related to amino acid concentrations and requirements in the intestinal lumen [16]. The mechanisms by which *L. reuteri* LR1 regulates intestinal amino acid transporter expression require further investigation.

3.4 Effects of *L. reuteri* LR1 on Intestinal Fatty Acid Synthesis in Weaned Piglets Dietary fat consists primarily of triglycerides (TG), which are digested into fatty acids and monoglycerides in the intestine, absorbed by intestinal epithelial cells, and resynthesized into TG that is packaged into chylomicrons for transport to peripheral tissues [17]. Fatty acid synthesis occurs

in the cytoplasm and involves numerous key transporters and enzymes. For example, fatty acid binding proteins (FABP) catalyze the entry of free fatty acids into cells, ACC is the rate-limiting enzyme in the first stage of fatty acid synthesis, and FASN is a key enzyme catalyzing fatty acid chain synthesis. This study showed that *L. reuteri* LR1 significantly upregulated mRNA expressions of I-FABP, FABP3 in the duodenum, and FABP3 in the jejunum, participating in fatty acid transport. Previous reports indicated that *Bacillus subtilis* upregulated mRNA expressions of ACC and FASN in porcine subcutaneous adipose tissue [18]. Similarly, our study found that mRNA expressions of both enzymes were significantly increased in the duodenum of *L. reuteri* LR1-treated piglets, suggesting that *L. reuteri* LR1 promotes fatty acid transport and expression of genes related to de novo intracellular fatty acid synthesis.

SREBP1 and PPAR are important transcription factors and nuclear genes regulating fatty acid synthesis [19]. Studies have found that *Lactobacillus johnsonii* increased PPAR mRNA expression in broiler liver while downregulating SREBP1c mRNA expression [20]. Our results were similar: *L. reuteri* LR1 significantly increased PPAR mRNA expression in all three intestinal segments, while SREBP1 mRNA expression showed no significant differences in the anterior small intestine but was decreased in the ileum. We therefore speculate that PPAR is the most important regulatory factor for intestinal fatty acid synthesis in *L. reuteri*-treated piglets.

Small intestinal tissue adapts to dietary fat content through changes in fat absorption capacity, particularly through coordinated induction of fatty acid binding proteins [21]. Most fat digestion and absorption occurs in the anterior small intestine [13]. As mentioned earlier, *L. reuteri* LR1 increased CCK mRNA expression, causing gallbladder contraction and bile release, as well as pancreatic digestive enzyme secretion. Fat entering the anterior small intestine is emulsified by bile salts into chylomicrons and digested by pancreatic lipase into fatty acids and other products. To accommodate increased fatty acid content, mRNA expressions of I-FABP and FABP3 responsible for fatty acid transport were upregulated in the anterior small intestine, allowing more fatty acids to enter intestinal epithelial cells for TG synthesis under PPAR regulation with participation of key enzymes such as ACC and FASN. Conversely, the posterior small intestine adapted to reduced fatty acid content, which explains why *L. reuteri* LR1 had minimal effects or even exerted negative regulation on fatty acid synthesis-related gene expression in the ileum.

This study also demonstrated that *L. reuteri* LR1 affected intestinal glucose transport in piglets. Faseleh et al. [22] reported that lactic acid bacteria upregulated mRNA expressions of glucose transporters including GLUT2, GLUT5, SGLT1, and SGLT4 in broiler intestine. Other studies showed that pretreatment with *L. plantarum* followed by *Escherichia coli* challenge significantly upregulated SGLT1 mRNA expression in porcine intestinal epithelial cells [14]. Our study found that *L. reuteri* LR1 increased SGLT1 mRNA expression in the jejunum and ileum but decreased GLUT2 mRNA expression in the jejunum, par-

tially differing from previous studies, possibly due to different bacterial strains and experimental subjects.

The mTOR signaling pathway plays an important role in regulating protein synthesis [23]. This study found that antibiotics upregulated mTOR expression in the jejunum and ileum, suggesting that antibiotics may promote protein synthesis by activating the mTOR signaling pathway, though this mechanism requires further verification. We also found that antibiotics promoted mRNA expressions of SGLT1 and SGLT3 in the duodenum and increased mRNA expressions of amino acid transporters y LAT1, CAT1, and PepT1 in the jejunum. Studies have shown that dietary enramycin supplementation promotes mRNA expressions of SGLT1 and PepT1 in piglet intestine [24], consistent with our results and suggesting that antibiotics can facilitate absorption of small peptides and amino acids in the jejunum.

Normal intestinal mucosal structure is a prerequisite for normal digestive and absorptive function. Generally, increased villus height and surface area enlarge the contact area between transporters and nutrients, facilitating nutrient absorption [25]. Our previous study found that *L. reuteri* LR1 improved intestinal morphology, manifested as greater ileal villus height and increased villus height-to-crypt depth ratios in the jejunum and ileum [7]. Zhou [15] suggested that the beneficial effects of lactobacilli on intestinal villus growth are related to upregulation of intestinal nutrient transporter mRNA expression. Additionally, upregulation of amino acid transporters may contribute to intestinal morphological integrity and nutrient absorption, thereby benefiting animal growth [26]. Therefore, the regulatory effects of *L. reuteri* LR1 on mRNA expression of intestinal nutrient transporters provide further evidence that *L. reuteri* LR1 improves intestinal mucosal morphology and consequently facilitates nutrient absorption.

Conclusion

Dietary supplementation with 5.0×10^1 CFU/kg *Lactobacillus reuteri* LR1 promotes intestinal nutrient digestion and absorption in weaned piglets. *Lactobacillus reuteri* LR1 and antibiotics exhibit similar regulatory effects on mRNA expression of intestinal nutrient transporters, promoting amino acid absorption and fatty acid synthesis. Therefore, *L. reuteri* LR1 shows promise as an antibiotic alternative for improving piglet growth performance.

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Note: Figure translations are in progress. See original paper for figures.