Supplementary Materials

Lactobacillus reuteri **JCM 1112 ameliorates chronic acrylamide-induced glucose metabolism disorder via bile acids-TGR5-GLP-1 axis and modulates intestinal oxidative stress in mice**[1]

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Supplementary Methods

1. UPLC-MS/MS analysis

UPLC-MS/MS was performed using an Agilent 1290 Infinity LC system (Agilent, Santa Clara, USA) coupled with an AB SCIEX QTRAP 5500 mass spectrometer (AB Sciex, Framingham, USA). The Agilent 1290 Infinity LC system was equipped with an ACQUITY UPLC BEH C18 column (100 mm × 2.1 mm i.d., 1.8 μm; Waters, Milford, USA). The column oven temperature was set to 45 °C. The mobile phases consisted of 0.1% formic acid in water (A) and methanol (B). The injection volume was 2 μL and the flow rate was 0.25 mL/min. The gradient elution conditions of mobile phases were as follows: 50% to 70% of B at 0-7 min; 70% to 90% of B at 7-15 min; 90% B maintained at 15-17 min; 90% to 60% of B at 17-17.1 min, 60% B maintained at 17.1-20 min. The MS analysis was performed in negative ion mode using an AB SCIEX QTRAP 5500 system equipped with electron spray ionization (ESI). The ESI source conditions were set as follows: source temperature, 550 ℃; ion source Gas1 (Gas1), 55 psi; ion source Gas2 (Gas2), 55 psi; curtain gas (CUR), 40 psi; and ion sapary voltage floating (ISVF), -4500 V. The MRM mode was used for the acquisition of MS data. In addition, equal volumes of all samples were mixed to generate quality control (QC) samples, which were used to evaluate the stability and repeatability of the method.

2. Reverse transcription and qPCR assay

Reverse transcription of the total RNA (1 μg) was performed using HiScript II Q RT SuperMix Kit (Vazyme). qPCR was run in a CFX96 Touch™ Real-Time PCR System (BioRad, Hercules, USA) using ChamQ SYBR qPCR Master Mix Kit (Vazyme). The reaction conditions were set as follows: 95 °C for 30 s, 40 cycles of 10 s at 95 °C and 30 s at 60 °C, followed by melting curve analysis.

Supplementary tables

Table S1 The compositions of SPF-grade standard diet

	Component	**Content**		**Component**	**Content**		**Component**	**Content**
Vitamin	Vitamin A (IU/kg)	20000.00	**Mineral**	Sodium (g/kg)	2.83	**Amino acids**	Methionine+Cysteine (g/kg)	8.00
	Vitamin D (IU/kg)	1667.00		Magnesium (g/kg)	2.77		Lysine (g/kg)	13.90
	Vitamin E (mg/kg)	182.00		Potassium (g/kg)	8.20		Tryptophan (g/kg)	2.50
	Vitamin K (mg/kg)	8.00		Copper (mg/kg)	12.41		Arginine (g/kg)	12.00
	Vitamin B1 (mg/kg)	20.23		Iron (mg/kg)	158.60		Leucine (g/kg)	17.60
	Vitamin B2 (mg/kg)	20.00		Manganese (mg/kg)	88.10		Isoleucine (g/kg)	10.50
	Vitamin B6 (mg/kg)	15.00		Zinc (mg/kg)	50.70		Threonine (g/kg)	8.80
	Vitamin B12 (mg/kg)	0.03		Selenium (mg/kg)	0.20		Valine (g/kg)	11.90
	Niacin (mg/kg)	70.00		Iodine (mg/kg)	0.90		Histidine (g/kg)	5.60
	Pantothenic acid (mg/kg)	25.00					Phenylalanine+tyrosine (g/kg)	16.80
	Biotin (mg/kg)	0.30						
	Choline (mg/kg)	1250.00						
	Folic acid (mg/kg)	10.00						

Table S2 the primer sequences for qPCR assay

Gene name	Primer sequence (5'–3')	Reference
TGR5-F	TATGGAGCCGGAACCATCAG	Liu et al., 2022
TGR5-R	GGCAAGCAGGGAAAGGAAAC	
FXR-F	CCTCCTCGTCTTACTATTCC	
FXR-R	GTCACAGGCATCTCTGATAC	
β-actin-F	GGCTGTATTCCCCTCCATCG	
β-actin-R	CCAGTTGGTAACAATGCCATGT	
PG-F	GATCATTCCCAGCTTCCCAG	Trabelsi et al., 2015
PG-R	CTGGTAAAGGTCCCTTCAGC	

Reference

Liu, X., Zhang, Y., Li, W., Zhang, B., Yin, J., Liuqi, S., Wang, J., Peng, B., Wang, S., 2022. Fucoidan ameliorated dextran sulfate sodium-induced ulcerative colitis by modulating gut microbiota and bile acid metabolism. J Agric Food Chem. 70, 14864–14876.

Trabelsi, M. S., Daoudi, M., Prawitt, J., Ducastel, S., Touche, V., Sayin, S. I., Perino, A., Brighton, C. A., Sebti, Y., Kluza, J., Briand, O., Dehondt, H., Vallez, E., Dorchies, E., Baud, G., Spinelli, V., Hennuyer, N., Caron, S., Bantubungi, K., Caiazzo, R., … Lestavel, S. (2015). Farnesoid X receptor inhibits glucagon-like peptide-1 production by enteroendocrine L cells. Nat Commun. 6, 7629.

Supplementary figures

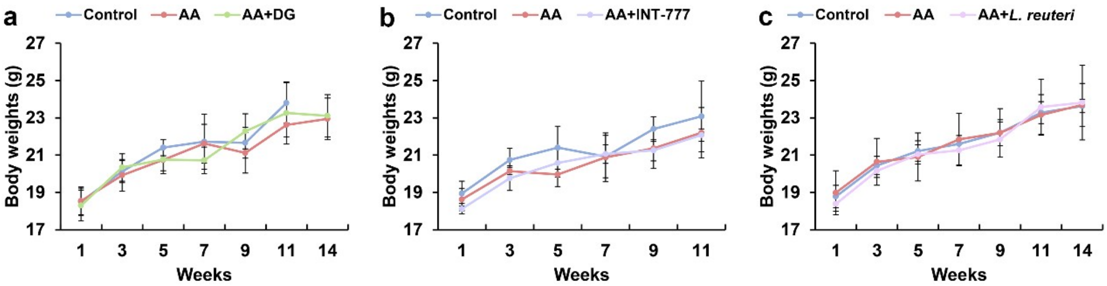

Fig. S1 Body weights of mice in animal experiment I (a), II (b) and III (c).

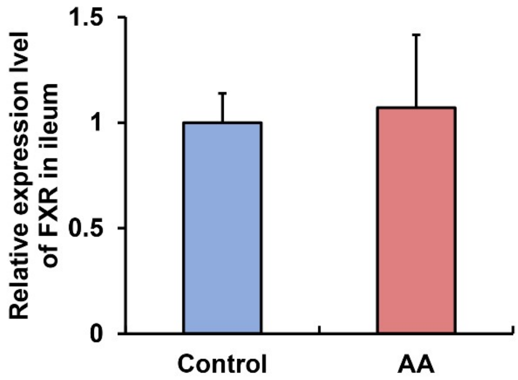

Fig. S2 The effect of chronic low-dose AA exposure on ileum FXR gene expression in mice.

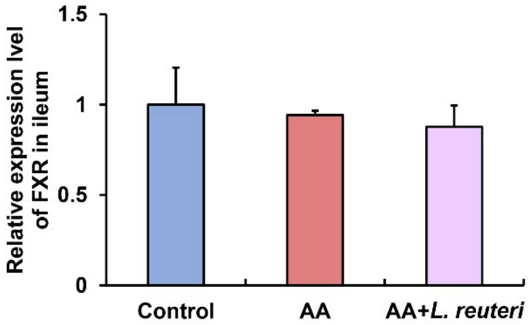

Fig. S3 The effect of *L. reuteri* on ileum FXR gene expression in chronic low-dose AA-exposed mice.