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Dietary Resistant Starch Regulates Bile Acid Metabolism by Modulating the FXR/LRH-1 Signaling Pathway in Broilers

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Abstract: This study aimed to investigate the effects of dietary corn-resistant starch on the bile acid metabolism of broilers. In total, 80, 1-day-old male broilers were randomly distributed into two groups fed either the basic normal corn–soybean diet or a diet supplemented with 40 g/kg of corn-resistant starch. The results showed that dietary supplementation of 4% corn-resistant starch increased the F/G during the periods from 21 to 42 d. Resistant starch supplementation reduced the lipid levels in plasma, and the contents of total bile acids were increased with the altered bile acid profile in the ileum. A diet with corn resistant starch decreased the enzyme contents of the classical pathway of bile acid synthesis and activated the signaling pathway of FXR/LRH-1 in the liver. A decreased abundance of *Clostridium cluster XIVa* was found in the ileal digesta of the resistant starch group, and its abundance was negatively correlated with the level of lithocholic acid. In summary, the RS was effective at reducing broiler plasma and liver lipid levels, which was probably due to the change in bile acid synthesis and reabsorption capacities. These findings provided a unique landscape of the relationship between bile acid metabolism and resistant starch in broilers.

Keywords: resistant starch; bile acid metabolism; microorganism; FXR/LRH-1

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1. Introduction

Starch accounts for approximately 40% of the nutrient requirements for broilers, and provides more than 50% of the metabolic energy, which plays an important role in maintaining glucose and lipid metabolism [1], as well as the stability of gut microbiota in poultry [2]. Starch can be classified into three types, which are usually named rapidly digestible starch, slowly digestible starch, and resistant starch (RS) [3]. Resistant starch is a typical dietary fiber and is a form of high-amylose maize starches, which could be resistant to enzymatic digestion in the small intestine. As a fermentable dietary fiber, RS can be classified into five sub-types, depending on the source and physical structure of the starch [4]. Resistant starch, especially RS2, increases feelings of fullness and provides minimal energy [5], which helps to reduce overall energy intake and contributes to the prevention of fat deposition.

The small intestine is the primary site where resistant starch exerts its effects. Previous studies have revealed that RS2 can enhance intestinal integrity and improve gut microbiota diversity [6]. Due to RS2's advantageous effects on microbiota composition and metabolism, it is commonly added to animal feeds to improve the growth performance of ruminants [5].

The content of RS in poultry diets is typically low, usually below 5%, making it difficult to meet the growth requirements of poultry. The supplementation of RS is a widely recognized and effective method to address this issue. For example, a diet with RS increased the abundance of *Lactobacillus* in the small intestine and promoted the diversity and stability of microbiota in weaned piglets [7]. Likewise, the supplementation of 4% corn resistant starch (one of RS2) raised the abundance of *Bacteroidetes*, but decreased the abundance of *Firmicutes* in broiler cecum [6]. Our previous studies have found that a diet with 4% corn resistant starch effectively alters the composition of intestinal microbiota, particularly increasing the abundance of bile acid metabolism-related bacterial communities [6].

Bile acids are bioactive molecules that have a positive effect on the digestion and absorption of dietary fats and interact with the gut microbiota, influencing the composition and metabolic activity of these microorganisms [8,9]. Bile acids are mainly synthesized through the enterohepatic circulation and are the main products of cholesterol metabolism [10]. Briefly, the cholesterol could be synthesized into cholic acid (CA) by cholesterol 7α-hydroxylase (CYP7A1) and synthesized into chenodeoxycholic acid (CDCA) by sterol 27-hydroxylase (CYP27A1); these are also named primary bile acid [11]. The primary bile acid enters the intestine via the biliary duct, and finally generates secondary bile acid under the reaction of microorganisms [10]. The transportation of bile acids in the tissues mainly depends on bile acid receptors on the cell membrane, and the bile acid transporters in different organs are not uniform [12]. Specifically, the bile salt export pump (BSEP) and multidrug resistance-related protein 2 (MRP2) transport primary bile acids to the bile duct, which ultimately enter the intestine. Small molecule bile acids outside of the liver are reabsorbed and taken back into the liver through Na+-dependent taurocholate cotransporting polypeptide (NTCP) and organic anion-transporting polypeptide 1 (OATP1) [13]. Intestinal bile acids enter ileum cells via apical sodium-dependent transporter (ASBT), then are transported to the basolateral membrane by the ileum bile acid binding protein (IBABP) and activate organic solute transporter (Ost α/β) to enter the vein, and finally return to the liver through the enterohepatic circulation [14]. In addition, the synthesis and transport of bile acids are also regulated by the bile acid transporter and Farnesoid X receptor (FXR) [15]. Previous studies have revealed that FXR activation protects against abnormal lipid metabolism via bile-acid-dependent reductions in lipid absorption [16].

The intestinal microbiota regulates the composition and proportion of bile acids, which in turn influences the organization of various microbial communities within the gut [17]. An in vitro test found that bile acids altered the presence of membrane structures of *Lactobacillus*, which modulated the activity of beta-galactosidase [18]. Additionally, it was observed in a rat model that a diet supplemented with cholic acid led to an increase in the population of *Firmicutes* and *Bacteroidetes*, two major bacterial phyla in the gut microbiota, which reshaped the composition of the gut microbiota [17]. Therefore, RS-mediated bile acid metabolism is an important factor for animal growth and metabolism, while the molecular mechanism needs further exploration. Thus, the objective of this research was to assess the impact of corn RS on the characteristics of bile acid metabolism in broiler.

2. Materials and Methods

2.1. Animals and Experimental Design

In this trial, at the age of 1 d, in total, 80 male Ross-308 broilers (Panchu Broiler Breeding Co., Ltd., Nanjing, China) were allocated randomly into two groups, with each group consisting of five replicate cages (the specific size of the cage is 1.3 m $\times$ 0.9 m $\times$ 0.5 m) and each replicate cage having eight broilers: (i) the NC group, which was fed a normal commercial broiler corn–soybean diet; (ii) the RS group, which was fed the diet supplementation with 20% starch (Type II RS with a purity level of 20%; corn starch with a purity level of 79%), where the feed was provided in pellet form. Table 1 displays the nutritional composition of each diet. The entire experimental period consisted of two feeding stages (1–21 d and 22–42 d), with the first week of rearing maintaining a room temperature of

33 °C. After that, a gradual decrease in temperature commenced, with a reduction of 3 °C per week. This progressive process continued until the desired final temperature of approximately 26 °C was achieved. From the beginning to the end of the experiment, the feed and water were supplied with free access for broilers, the humidity of the broiler coop was adjusted according to the optimal parameters for broiler rearing, and a daily light time was set for 23 h; the experimental period lasted for 42 days.

Table 1. Ingredient composition and nutrient contents of experimental diets.

Item [1]	1–21 d		22–42 d	
	NC	RS	NC	RS
Ingredients, %				
Soybean meal	31.50	28.15	25.00	24.50
Corn	57.00	36.50	61.30	38.00
Soybean oil	3.10	2.20	4.10	4.50
Limestone	1.20	1.20	1.40	1.40
Starch (20% resistant starch)	-	20	-	20
Corn gluten meal	3.40	8.15	4.60	8.00
L-Lysine hydrochloride	0.34	0.34	0.30	0.30
Dicalcium phosphate	2.00	2.00	1.70	1.47
Salt	0.30	0.30	0.30	0.30
DL-Methionine	0.15	0.15	0.08	0.07
Zeolite powder	0.01	0.01	0.22	0.56
Premix [2]	1.00	1.00	1.00	1.00
Calculated nutrient levels (%)				
Arginine	1.27	1.18	1.11	1.06
Methionine	0.50	0.51	0.41	0.41
Lysine	1.21	1.12	1.04	1.00
Methionine + cysteine	0.86	0.85	0.75	0.72
Threonine	0.83	0.81	0.75	0.74
Non-phytate phosphorus	0.46	0.45	0.40	0.35
Calcium	1.00	1.00	0.98	0.89
Crude protein	21.33	21.00	19.49	19.40
Metabolizable energy (MJ/kg)	12.52	-	13.00	-
Analyzed nutrient levels (%)				
Starch [3]	51.25	51.34	52.22	51.84
RS [3]	3.03	7.33	3.27	7.39
Crude protein	20.91	20.61	18.85	18.37
Calcium	1.01	0.96	0.99	0.92
Available phosphorus	0.47	0.48	0.41	0.38

[1] NC, a basic normal corn–soybean diet; RS, 4% corn resistant starch. [2] Premix provided per kilogram of diet: thiamine, 2.18 mg; choline pyridoxine HCl, 3.95 mg; chloride, 390 mg; biotin, 0.038 mg; riboflavin, 7.95 mg; vitamin K3, 1.28 mg; vitamin E, 22 IU; vitamin A, 11,950 IU; vitamin D, 2450 IU; vitamin B12 (cobalamin), 0.013 mg; nicotinamide, 38 mg; pantothenate acid (D-Ca pantothenate), 14.5 mg; folic acid, 1.0 mg; Se, 0.25 mg; Zn, 58 mg; Fe, 78 mg; Mn, 117 mg; I, 1.05 mg; Cu, 8.2 mg. [3] The starch and resistant starch content in the diets were determined using starch (Solebro, China) and resistant starch (Megazyme, Dublin, Ireland) content assay kits, respectively.

2.2. Growth Performance and Sample Collection

At 21 d and 42 d of the experiment, the growth performance indexes were calculated. Then, two broilers with a body weight similar to the mean body weight were selected and weighed in each replicate for the sample collection on 42 d. Using a syringe containing EDTA to collect 5 mL of plasma, the samples were centrifuged at 3500× *g* for 15 min, and then using a pipette, we accurately aspirated 1 mL of the supernatant and stored it at −20 °C in the refrigerator for further determinations. An appropriate amount of liver tissue was collected and stored at −80 °C.

The intestinal lumen of the ileum was aseptically dissected using sterile scissors, and the collected ileum digesta were used to determine the bacterial activity. Subsequently, sterile microscope slides were used to scrape the ileum mucosal tissue for gene expression analysis.

2.3. Plasma and Liver Lipid Metabolism

In accordance with the commercial kit instructions, we used about 30 mg of the liver tissue sample and 100 μL of the plasma sample to measure the markers related to lipid metabolism, mainly including total triglyceride (TG, A110–2), low-density lipoprotein (LDL-C, A113-2), high-density lipoprotein (HDL-C, A112-2), total cholesterol (TC, A111-2), and total bile acids (TBA, E003-2-1) (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

2.4. Contents of Bile Acid Synthase in Liver

The contents of the crucial classical pathway and alternative pathway of bile acid synthesis enzymes in liver were detected using commercial kits (CYP7A1 (H461-1), CYP8B1 (H518-1), CYP27A1 (H519-1), and CYP7B1 (H349-1)) (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

2.5. Targeted Metabolomic Analysis of Ileum Bile Acid

Briefly, 25 mg of ileal digesta was weighed and mixed evenly with 100 μL of 0.1% extraction solution (methanol: acetonitrile: water = 2: 2: 1). Samples were then subjected to grinding at 35 Hz for 4 min, followed by a 5 min of ultrasonication in an ice water bath, and this process was repeated twice. Next, the sample was left at −40 °C for one hour, and then centrifuged at 12,000 rpm for 15 min at 4 °C. The bile acid concentration in the supernatant was then measured using ultra-performance liquid chromatography–triple quadrupole mass spectrometry. UHPLC-PRM-MS/MS technology has become an important method for the determination of bile acids due to its high sensitivity, good specificity, wide quantitative range, and simple processing methods. Detecting and analyzing the bile acid profile of ileum digesta can intuitively reflect the impact of corn RS on the bile acid profile.

2.6. RT-PCR Analysis

In an enzyme-free environment, total RNA was isolated using the commercial kits (DP419). Using biophotometer (NanoDrop ND 2000, Thermo, Waltham, MA, USA) and agarose gel electrophoresis to evaluate the RNA quality. RNA concentration was determined using Nano Drop ND 2000 (Thermo, USA). Fast King Super Mix Kit (KP118) was then used to synthesize cDNA. Super Real SYBR Green Kit (FP205) and StepOnePlus (Applied Biosystems, Carlsbad, CA, USA) devices were used for real-time PCR. The entire reaction system was 20 ul, including 10 μL of SYBR Premix, 0.5 μL of ROX, 1.5 μL of cDNA, 7.2 μL of dd water, and 0.8 μL of primer (0.4 μL of each primer). The specific program parameters were as follows: 95 °C for 20 s, 95 °C for 5 s followed by 40 cycles for denaturation, and annealing and extension at 60 °C for 50 s. The primer sequences utilized for the study can be found in Table 2. To determine the mRNA expression levels, the $2^{-\Delta\Delta Ct}$ method was employed for calculations [19].

2.7. Extraction of Bacterial DNA

Ileal digesta samples were subjected to DNA extraction using the Mobio PowerLyzer® PowerSoil® DNA isolation kit (Mibio, 12888-50, Carlsbad, CA, USA) as instructed by the manufacturer. DNA concentration was determined using the Qubit double-stranded DNA high-sensitivity (dsDNA HS) assay kit (Yeasen, 12642ES60, Nanjing, China) and subsequently stored at −20 °C for further analysis. The amplified products were purified from the agarose gel (electrophoresis conditions: 1.5% agarose gel, 150 V, and 30 min) using the commercial purification kit (TIANGEN, DP203, Hangzhou, China) and then cloned into the DH5α vector (TaKaRa, 9126, Osaka, Japan). Plasmid DNA was extracted using the plasmid kit (ABclonal, RK30103, Nanjing, China). All bacteria were cultured anaerobically or aerobically in LB broth at 37 °C for 12 h, and the primers of all the bacteria are listed in Table 3.

Table 2. Gene-specific primers of related genes for broilers.

Gene [1]	GenBank Number	Primer Sequence (5′ → 3′)	Products (bp)
BSEP	XM_004942757.3	Forward: GGTTCATCCTGCAGAGACATC Reverse: CGCTTCTGGAATGTTTGGGG	129
MRP2	XM_025151804.1	Forward: TCATCAAACAGGTGCTGGCT Reverse: GGGTCCCAGGTGACGATGT	142
OATP1	NM_001318449.1	Forward: CTCTGTCACCTTGGGGCAATGTC Reverse: AACTCTGGCTGAACGCATCTGTAC	150
NTCP	XM_015287931.1	Forward: AGACAGGGATGGTTGTGCTT Reverse: CTGAGGGGAGATGGTGATGT	106
ASBT	NM_001319027.1	Forward: GGGGATGATGCCACTCTGTC Reverse: CCAATGCTGTCGTAGGGGAG	84
IBABP	XM_015293653.2	Forward: TCGGTCTCCCTGCTGACAAGATC Reverse: AGTCGTGGTGCGTCCTCCTG	119
Ost α	NM_001277697.1	Forward: CGTTCCATGATGGTGGTGGA Reverse: ACCATGGGCACGTCCTTTAG	134
Ost β	XM_025153901.1	Forward: GTCCTAAAGGCACCTTGGCT Reverse: GTGGTCCCACAAGTGACACA	254
FXR	NM_204113.2	Forward: AGTAGAAGCCATGTTCCTCCGTT Reverse: GCAGTGCATATTCCTCCTGTGTC	182
SHP	NM_001030893.2	Forward: GAGAACTGGCTTTGCGTGTG Reverse: AACTCAGTCTGCTCTGCGTC	235
LRH-1	NM_205078.1	Forward: TTAAGCGGACCGTCCAGAAC Reverse: GCATTTTTGGAACCGGCAGT	110
FGF19	NM_204674.2	Forward: GCTTCATCCTGCACCGTTTG Reverse: CGATCCCTCCCTGCAAGAAC	100
FGFR4	XM_015293864.2	Forward: TCATCGGGAAAGTCCAGCAC Reverse: CCAGCTTTTCTCGGGGGAAT	133
β-actin	NM_205518.1	Forward: ATCCGGACCCTCCATTGTC Reverse: AGCCATGCCAATCTCGTCTT	120

[1] ASBT, apical sodium-dependent bile acid transporter; BSEP, bile salt export pump; Ost α, organic solute transporter α; MRP2, multidrug resistance-associated protein 2; Ost β, organic solute transporter β; OATP1, organic anion transporting polypeptides 1; IBABP, ileal bile acid binding protein; NTCP, Na+/taurocholate cotransporting polypeptide; FXR, farnesoid X receptor; SHP, small-heterodimer partner; LRH-1, liver receptor homolog-1; FGF19, fibroblast growth factor 19; FGFR4, fibroblast growth factor receptor 4.

Table 3. Primers used for real-time PCR of the microorganisms in the ileum.

Gene	Primer Sequence (5′ → 3′)	Products (bp)	Reference
Bifidobacillus	Forward: GCGTGCTTAACACATGCAAGTC Reverse: CACCCGTTTCCAGGAGCTATT	126	[20]
Bacteroides	Forward: CTGAACCAGCCAAGTAGCG Reverse: CCGCAAACTTTCACAACTGACTTA	140	[21]
Lactobacillus	Forward: AGCAGTAGGGAATCTTCCA Reverse: CACCGCTACACATGGAG	341	[22]
Clostridium cluster XI	Forward: ACGCTACTTGAGGAGGA Reverse: GAGCCGTAGCCTTTCACT	55	[23]
Clostridium cluster XIVa	Forward: GAWGAAGTATYTCGGTATGT Reverse: CTACGCWCCCTTTACAC	59	[24]
Escherichia coli	Forward: CATGCCGCGTGTATGAAGAA Reverse: CGGGTAACGTCAATGAGCAAA	95	[20]

2.8. Statistical Analysis

The statistical analysis of this trial was performed using SPSS (version 22.0), and each replicate served as an individual experimental unit. All data were presented as mean ± standard error (SE). To identify significant differences between the two groups, the *t*-test was utilized as the statistical analysis method. p-values < 0.05 were considered significant.

3. Results

3.1. Growth Performance

As shown in Table 4, compared with the NC group, the dietary supplementation of 4% corn RS significantly increased the F/G ($p < 0.05$), had a tendency to decrease the ADG during the periods from 21 to 42 d, and had a tendency to increase the F/G during the periods from 1 to 42.

Table 4. Effects of corn RS on the growth performance of broilers.

Items [2]	Treatment [1]		SEM	*p*-Value
	NC	RS		
1–21 d				
ADG (g)	36.35	36.15	1.25	0.962
ADFI (g)	51.86	51.11	0.31	0.390
F/G	1.43	1.41	0.06	0.488
21–42 d				
ADG (g)	73.53	70.50	2.05	0.066
ADFI (g)	140.60	146.65	4.56	0.544
F/G	1.91	2.09 *	0.03	0.033
1–42 d				
ADG (g)	54.94	53.3	1.60	0.542
ADFI (g)	96.23	98.88	2.18	0.640
F/G	1.75	1.86	0.03	0.082

[1] NC, a basic normal corn–soybean diet; RS, 4% corn resistant starch, (n = 5). [2] ADG, average daily gain; ADFI, average daily feed intake; F:G, feed:gain ratio. * Represents significant differences ($p < 0.05$).

3.2. Plasma and Liver Lipid Levels

As shown in Figure 1, compared with the control group, the concentrations of TG, TC, LDL-C, and HDL-C in plasma were reduced ($p < 0.05$) in the RS group (Figure 1A). In addition, the supplementation of corn RS also decreased ($p < 0.05$) the TC levels in the liver with no changes in TG (Figure 1B). These data suggest that adding corn RS decreased lipid accumulations in broilers.

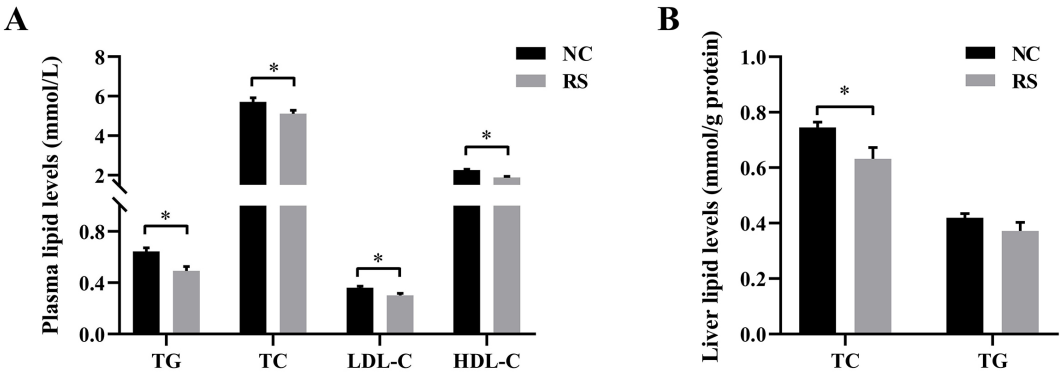

Figure 1. Effects of corn RS supplementation on the plasma and liver lipid levels of broilers on d 42. (**A**) Plasma lipid levels. (**B**) Liver lipid levels. NC, a basic normal corn–soybean diet; RS, 4% corn resistant starch. All data are represented by the mean ± standard error (n = 5). * Represents significant differences ($p < 0.05$).

3.3. Contents of Total Bile Acids

Due to the important functions of bile acids in lipid metabolism, the contents of TBAs were measured in the liver and ileum. In comparison with the NC group, the diet with 4% corn RS increased ($p < 0.05$) TBA levels in both the liver and ileum of broilers at 42 d of age (Figure 2). Therefore, RS might regulate lipid metabolism through its regulatory functions on TBA.

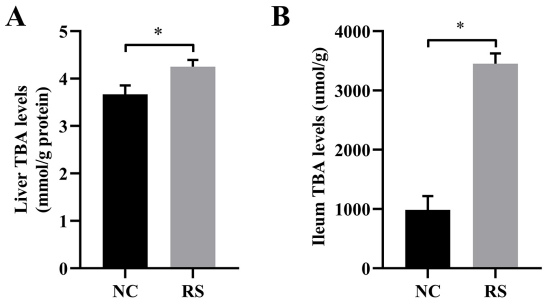

Figure 2. Effects of corn RS supplementation on the contents of total bile acids in the liver and ileum. (**A**) Contents of total bile acids in the liver, and (**B**) contents of total bile acids in the ileum. NC, a basic normal corn–soybean diet; RS, 4% corn resistant starch. All data are represented by the mean ± standard error (n = 5). * Represents significant differences ($p < 0.05$).

3.4. Ileum Bile Acid Metabolism

The bile acid profile in the ileum was further investigated to show the effects of RS supplementation on bile acid isoforms. Firstly, hierarchical cluster analysis showed that compared with the NC group, that fed a diet with 4% corn RS had increased ($p < 0.05$) contents of most bile acid isoforms in the ileum including chenodeoxycholic acid, hyodeoxycholic acid, and Taurolithocholic acid (Figure 3A). Also, contents of PBA, SBA, FBA, and CBA were increased ($p < 0.05$) in the RS group compared with those in the NC group (Figure 3B). In addition, Figure 3C revealed that the RS group increased ($p < 0.05$) the relative abundance of taurochenodexycholic acid, and taurochenodeoxycholic acid, and decreased the relative abundance of cholic acid, chenodeoxycholic acid, and 7-ketolithocholic acid in the top bile acids, which suggested that corn RS supplementation changed the ratio of bile acids in the ileum. In absolute content indexes, the RS group increased ($p < 0.05$) the contents of chenodeoxycholic acid, cholic acid, taurochenodexycholic acid, allocholic acid, 3-dehydrocholic acid, and 12-dehydrocholic acid in the ileum compared with those in the NC group (Figure 3D).

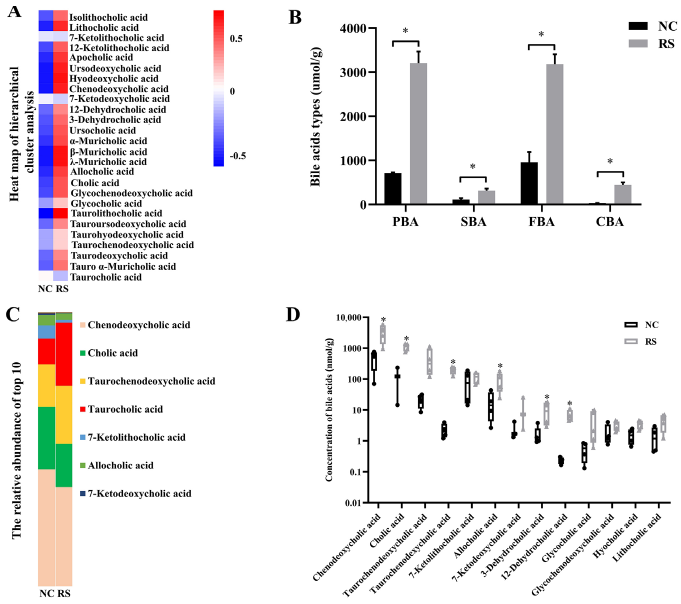

Figure 3. Effects of corn RS supplementation on the targeted metabolomic of ileum bile acids. (**A**) Heat map of hierarchical cluster analysis of bile acids. (**B**) The various types of bile acids. PBA = primary bile acids; SBA = secondary bile acids; FBA = free bile acids; CBA = conjugated bile acids. (**C**) The relative abundance of the top bile acids. (**D**) The bile acids with a content greater than 1 nmol/g. NC, a basic normal corn–soybean diet; RS, 4% corn resistant starch. All data are represented by the mean ± standard error (n = 5). * Represents significant differences ($p < 0.05$).

3.5. Contents of Liver Bile Acid Synthase

The contents of bile acid synthase in the liver were measured to show the condition of bile acids synthesis. Results showed that the contents of CYP7A1 and CYP8B1 were decreased ($p < 0.05$) while the contents of CYP27A1 were elevated ($p < 0.05$) in the RS group (Figure 4).

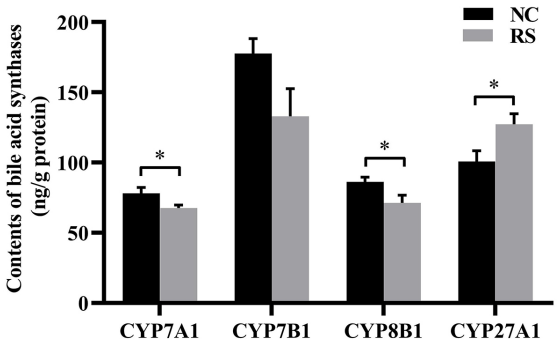

Figure 4. Effects of corn RS supplementation on the bile acid synthase in the liver of broilers. NC, a basic normal corn–soybean diet; RS, 4% corn resistant starch. All data are represented by the mean ± standard error (n = 5). * Represents significant differences ($p < 0.05$).

3.6. Gene Expression of Bile Acid Transporter

As shown in Figure 5, the supplementation of corn RS down-regulated ($p < 0.05$) the mRNA levels of *NTCP* and *OATP1* in the liver (Figure 5A), while no significant changes ($p > 0.05$) in the gene expression of *BSEP*, *MRP2*, *ABST*, *IBABP*, *Ost α*, and *Ost β* were found (Figure 5A,B).

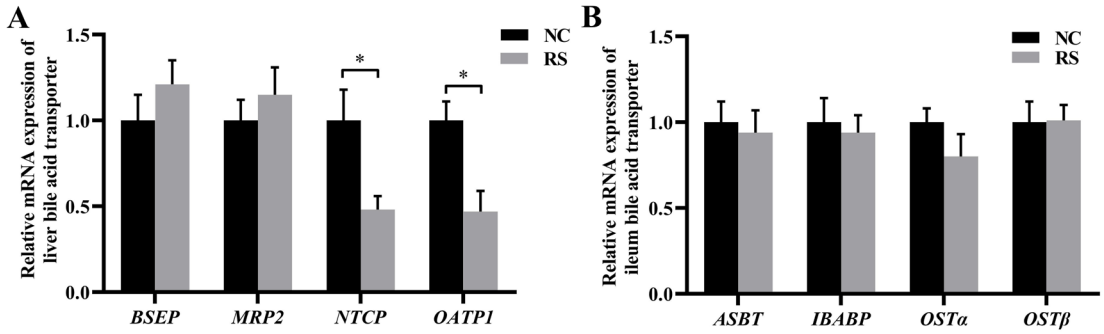

Figure 5. Effects of corn RS supplementation on the gene expression of bile acid transporter in the liver and ileum. (**A**) Relative mRNA expression of liver bile acid transporter. (**B**) Relative mRNA expression of ileum bile acid transporter. NC, a basic normal corn–soybean diet; RS, 4% corn resistant starch. All data are represented the mean ± standard error (n = 5). * Represents significant differences ($p < 0.05$).

3.7. FXR/LRH-1 and FXR-FGFR4 Signaling Pathway

As shown in Figure 6, a diet with 4% corn RS up-regulated ($p < 0.05$) the gene expression of *FXR* and down-regulated ($p < 0.05$) *LRH-1* in liver (Figure 6A). No difference ($p > 0.05$) in the expression of *SHP* was found. In addition, no significant difference ($p > 0.05$) was observed in the expression of the ileum's FXR-FGFR4 signaling pathway-related genes including FXR, FGF19, and FGFR4 (Figure 6B). All these findings indicated that the corn RS treatment's negative feedback inhibited the reabsorption and synthesis of bile acids through the activation of the FXR/LRH-1 signaling pathway in the liver.

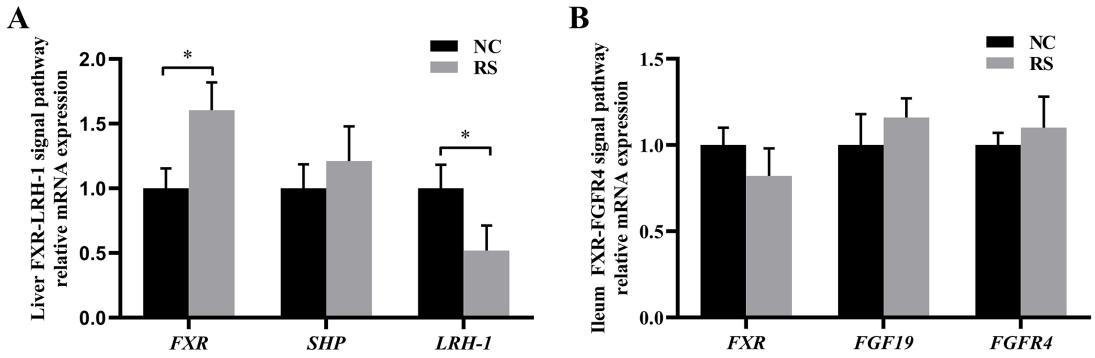

Figure 6. Effects of corn RS supplementation on the FXR/LRH-1 and FXR-FGFR4 signaling pathway. (**A**) The mRNA expression level of FXR/LRH-1 signaling pathway-related genes in the liver. (**B**) The mRNA expression level of FXR-FGFR4 signaling pathway-related genes in the ileum. NC, a basic normal corn–soybean diet; RS, 4% corn resistant starch. All data are represented by the mean ± standard error (n = 5). * Represents significant differences ($p < 0.05$).

3.8. Number of Specific Active Bacteria in Ileum

The abundance of gut microbiota was investigated to reflect the influence of RS supplementation on bile acid metabolism as bile acids directly affect microbiota composition. Through absolute quantification analysis, the results show that the RS supplementation decreased ($p < 0.05$) the abundance of *Clostridium cluster XI* (Figure 7A) in the ileum chyme compared with that in the NC group. Resistant starch did not affect ($p > 0.05$) the abundance of BSH-active bacteria (Figure 7B). In addition, a positive correlation was observed between *Bacteroides* abundance and hyocholic acid contents, and between *Lactobacillus* abundance and FBA, SBA, hyocholic acid, and glycochenodeoxycholic acid contents. In addition, the *Clostridium cluster XI* abundance and CBA, taurocholic acid, and taurochenodeoxycholic acid contents were negatively correlated. Moreover, the level of lithocholic acid was negatively correlated with the abundance of *Clostridium cluster XIVa.*

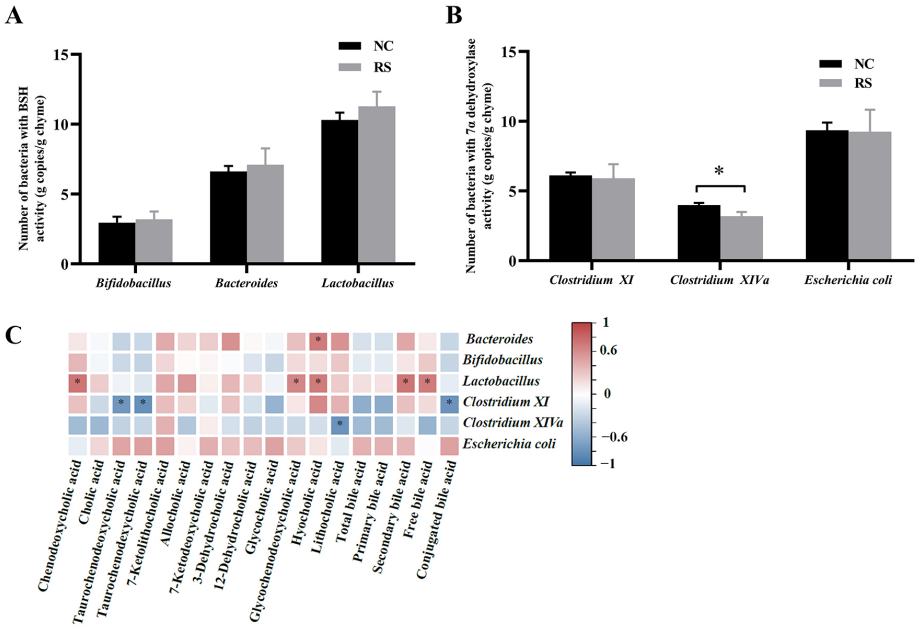

Figure 7. Effects of corn RS supplementation on the number of active bacteria in the ileum. (**A**) The number of bile salt hydrolase (BSH) active bacteria in the ileum. (**B**) The number of 7α dehydroxylase active bacteria in the ileum. (**C**) The correlation between bile acid levels and specific active bacteria. NC, a basic normal corn–soybean diet; RS, 4% corn resistant starch. All data are represented by the mean ± standard error (n = 5). * Represents significant differences ($p < 0.05$).

4. Discussion

Resistant starch provides a certain level of satiety and can alter intestinal motility, thereby influencing the feed intake and digestion of livestock and poultry [13]. Previous studies have revealed that an increase in the proportion of dietary RS leads to a reduction in the utilization efficiency of carbohydrates in the small intestine, accompanied by a decrease in the villus height and the activity of proteases in the small intestine [25], which explains why the diet with RS reduced feed the conversion rate during the periods from 21 to 42 d. Although current research has not improved the growth performance of broiler chickens, it provides a theoretical basis for precision feeding. A recent study found that a diet with 25% amylose significantly increased the average daily gain and carcass weight of pigs [26]. However, another study reported that there were no significant differences in growth performance after the supplementation of a diet with potato RS [27]. Also, corn RS-fed pigs showed declined feed conversion efficiency and body weight [28]. Interestingly, our results were partially consistent with those of Bergh and Liu, who reported that the diet with corn RS significantly reduced the feed intake/gain of broilers [25,29], and this may be attributed to the satiating effect of resistant starch and the differences in dietary nutrients levels.

Resistant starch, a specific type of dietary fiber, has gained recognition for its potential to prevent obesity, reduce fat accumulation, lower blood glucose levels, and improve insulin sensitivity [5]. Indeed, previous studies have found that a diet with corn RS significantly reduced the abdominal fat percentage [4]. The beneficial effects of RS are attributed to its ability to inhibit the digestion and absorption of carbohydrates, leading to a reduced release of glucose, and a reduction in fatty acid synthesis [30,31]. Additionally, the fermentation of RS in the ileum produces short-chain fatty acids and bile acids, which inhibits fatty acid synthesis and promotes fatty acid oxidation [32]. Physiological LDL-C, HDL-C, TC, and TG are commonly used as markers to assess plasma lipid levels, which serve as important indicators that indirectly reflect the metabolic status of liver and intestinal lipids [33]. In general, plasma lipids primarily originate from endogenous cholesterol and triglycerides synthesized by the liver, as well as exogenous chylomicron particles in the small intestine [32]. The supplementation of RS in rats could effectively reduce the plasma cholesterol levels [33]. Furthermore, eating RS-enriched flour for 2–12 weeks significantly suppressed serum TC and HDL-C levels [34]. Consistently, the present study indicated that dietary corn RS significantly reduced blood and liver lipid levels, especially cholesterol levels in broilers. The underlying mechanism may be related to hepatic bile acid metabolism and the enterohepatic circulation of bile acid. Therefore, this suggests that a diet with RS is effective in promoting the lipid metabolism of broiler chickens.

Bile acids are important metabolites of cholesterol, which are mainly divided into FBA and CBA according to their structures, and PBA and SBA according to their sources. Generally, PBAs (such as cholic acid and deoxycholic acid) are synthesized in the liver under the action of CYP and enter the intestine through the bile duct and enterohepatic circulation [35]. PBA and BSH cause a conjugated reaction by intestinal microbiota, and eventually generate SBA. Specifically, cholesterol metabolism into bile acids is an enzymatic reaction that occurs in liver cells, and there are two synthetic pathways: the classical pathway and the alternative pathway [36]. The classical pathway, also known as the neutral pathway, is the main pathway for bile acid synthesis, where the cholesterol in the liver is synthesized into cholic acid by CYP7A1, CYP8B1, and CYP27A1. It has been proposed that in the alternative pathway, also known as the acidic pathway, the cholesterol in the liver is synthesized into CDCA by CYP27A1 and CYP7B1, accounting for 18% of bile acid metabolism [37]. The present study demonstrated that bile acid isoforms with a content of more than 1 nmol/g in the ileum digesta are mainly composed of cholic acid, chenodeoxycholic acid, taurocholic acid, and taurochenodeoxycholic acid, and each accounted for approximately 42.72%, 22.82%, 15.46%, and 15.46% in the NC group, while the proportions of each bile acid isoforms in the RS group were 36.19%, 15.80%, 21.13%, and 23.08%. Obviously, the intervention of RS changes the proportion of bile acid subtypes in the ileum digesta. In terms of quantity, we observed a 6.72-fold increase in CDCA and a

6.21-fold increase in CA in the RS group, and the TBA levels in both the liver and ileum were increased in the RS group. Particularly, the contents of cholic acid and chenodeoxycholic acid were significantly increased in the ileum, and increased the contents of CYP27A1, but decreased the contents of bile acids synthetase including CYP7A1 and CYP8B1 in liver, which indicated that the classic pathway of bile acid production was attenuated and that the alternative pathway was activated. Meanwhile, the present study found that diets with corn RS also showed positive effects on the contents of 3-dehydrocholic acid and 12-dehydrocholic acid, which were mainly attributed to the microbial fermentation of cholic acid [38]. Additionally, the study found that lithocholic acid (LCA) cannot be absorbed by the intestine and is excreted with feces [39]. Therefore, those data showed that RS effectively increased the content of LCA in the ileum, indicating that RS promotes the excretion of bile acids and acts as a potential target for maintaining the steady state of bile acid metabolism, which provides new targets for improving intestinal bile acid metabolism in poultry.

Many factors play a role in the regulation of bile acid synthesis, transport, and metabolism. These include but are not limited to hormonal signals, genetic factors, microbiota, and various environmental stimuli, among which bile acid transporters and FXR play important roles [40]. Briefly, PBA enters the gallbladder through BSEP and MRP2 [41], which emulsify cholesterol and promote lipid metabolism. Then, the PBA of the gallbladder enters the intestine via the enterohepatic circulation and is fermented by microorganisms, and finally enters the intestinal epithelial cells via ABST [42]. With the transportation of IBABP, Ost α, and Ost β, bile enters the portal vein system and is ultimately reabsorbed by the bile acid transporters NTCP and OATP1 [11]. FXR is a member of the nuclear receptor superfamily and plays a crucial role in maintaining metabolic homeostasis, such as that in the digestion, absorption, and transport of liver lipids and glucose, as well as in energy metabolism, regulates bile acid metabolism and participates in enterohepatic circulation [40]. In addition, high levels of bile acid can trigger a FXR negative feedback regulatory mechanism that inhibits bile acid synthesis enzyme expression [43]. In the current study, dietary RS supplementation down-regulated the gene expression of *NTCP* and *OATP1*, but had no significant influence on the transport of bile in intestine, which indicated that the high levels of bile acids in liver were mainly caused by cholesterol metabolism rather than bile acid reabsorption. Notably, the signaling pathway of FXR/LRH-1 was activated via the treatment of dietary RS supplementation; as previous studies have demonstrated it as a bile acid sensor, FXR has a negative feedback action on the synthesis of bile acids, and prevents liver damage caused by high levels of bile acids [44]. Generally, after the bile acid level in the liver reaches a threshold, the bile acid will bind with FXR and activate SHP, resulting in a decreased expression of LHR-1 and ultimately indirectly inhibiting the expression of CYP7A1 [45]. Similarly, our results were partially consistent with the decrease in the classical pathway of bile acid synthesis. Furthermore,0 FXR is also widely present in the distal ileum and regulates the synthesis pathway of ileal bile acids through the FXR-FGFR4 signaling pathway [43]. Interestingly, dietary supplementation with RS in this experiment significantly altered the bile acid profile in the ileum, but the FXR-FGFR4 signaling pathway, which is involved in ileal metabolism, was not activated. This may be due to the greater role played by changes in the intestinal microbiota in this process, and the mechanisms involved need to be further explored in future research.

The intestine is the main place for the conversion of PBA into SBA [44]; with the catalysis of BSH enzyme activity bacteria (such as *Bifidobacillus*, *Bacteroides*, and *Lactobacillus*), dehydroxylation of cholic acid produces deoxycholic acid, whereas chenodeoxycholic acid is dehydroxylated into LCA. Although the abundance of *Bifidobacillus*, *Bacteroides*, and *Lactobacillus* did not change significantly in this experiment, there was a certain increasing trend in value, which may be attributed to the selection of strains, RS dosages, and species differences. In addition, we found that the abundance of BSH enzyme activity bacteria was positively correlated with the levels of secondary bile acids, which confirms that dietary RS indeed regulates ileal bile acid metabolism by changing intestinal microorganisms. Addi-

tionally, chenodeoxycholic acid is converted into ursodeoxycholic acid with the catalysis of 7-α-HSDH enzyme activity bacteria (including *Escherichia coli*, *Clostridium cluster XI*, and *Clostridium cluster XIVa*) [46]. It has been proposed that intestinal microbiota can alter the bile acid profile and regulate the intestinal FXR signaling pathway. However, bile acids have antimicrobial activity and can directly regulate the diversity of the gut microbiota in turn [47]. Therefore, the regulation between bile acids and the gut microbiota is mutual. The data presented here also revealed that supplementation with RS increases the overall level of bile acids in the ileum and reduces the abundance of *Clostridium cluster XIVa*, which may be related to the bacteriostatic effect of high levels of LCA. Lithocholic acid is a secondary bile acid derived from the metabolism of primary bile acids in the intestine and the secondary metabolism of intestinal microorganisms. It has an ability to inhibit cell growth and proliferation, which may be related to the interaction between LCA and receptors in the cell membrane or the regulation of the cell cycle [48]. Likewise, the correlation analysis revealed that the level of LCA was negatively correlated with the abundance of *Clostridium cluster XIVa*, which reveals insights into the specific interactions between resistant starch and gut microbiota providing, a unique landscape for further research. Interestingly, there is a certain positive correlation between LCA and other bacterial groups in this experiment. In addition, although there was no statistical influence in the abundance of other microorganisms, there was also an obvious correlation between these microorganisms and bile acids, especially a positive correlation between *Lactobacillus* and chenodeoxycholic acid, glycochenodeoxycholic acid, and hyocholic acid. Above all, dietary RS plays an important role in regulating the gut microbe structure and bile acid metabolism to some extent.

5. Conclusions

In summary, dietary supplementation with 4% corn RS effectively inhibits adipogenesis in broiler chickens, as primarily evidenced by the reduction in total triglyceride and total cholesterol levels in the liver and plasma. The underlying mechanism of this effect is likely attributed to the activation of the liver's FXR/LRH-1 signaling pathway, the enhancement of enzymatic activity, and the elevation in total bile acid levels. Based on the bidirectional effect of the enterohepatic circulation, the changes in microorganisms' abundance result in alterations in the bile acid profile in the ileum, leading to an increased concentration of total bile acids and a variety of secondary bile acids.

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References

1. Fasuan, T.O.; Asadu, K.C.; Anyiam, C.C.; Ojokoh, L.O.; Olagunju, T.M.; Chima, J.U.; Okpara, K.O. Bioactive and nutritional characterization of modeled and optimized consumer-ready flakes from pseudocereal (*Amaranthus viridis*), high-protein soymeal and modified corn starch. *Food Prod. Process. Nutr.* **2021**, *3*, 12. [CrossRef]
2. Svihus, B. Limitations to wheat starch digestion in growing broiler chickens: A brief review. *Anim. Prod. Sci.* **2011**, *51*, 583–589. [CrossRef]

3. Obadi, M.; Qi, Y.; Xu, B. High-amylose maize starch: Structure, properties, modifications and industrial applications. *Carbohydr. Polym.* **2023**, *299*, 120185. [CrossRef] [PubMed]
4. Guo, J.; Tan, L.; Kong, L. Impact of dietary intake of resistant starch on obesity and associated metabolic profiles in human: A systematic review of the literature. *Crit. Rev. Food Sci. Nutr.* **2021**, *61*, 889–905. [CrossRef]
5. Raigond, P.; Ezekiel, R.; Raigond, B. Resistant starch in food: A review. *J. Sci. Food Agric.* **2015**, *95*, 1968–1978. [CrossRef]
6. Zhang, Y.; Liu, Y.; Li, J.; Xing, T.; Jiang, Y.; Zhang, L.; Gao, F. Dietary resistant starch modifies the composition and function of caecal microbiota of broilers. *J. Sci. Food Agric.* **2020**, *100*, 1274–1284. [CrossRef]
7. Nielsen, T.S.; Lærke, H.N.; Theil, P.K.; Sørensen, J.F.; Saarinen, M.; Forssten, S.; Knudsen, K.E. Diets high in resistant starch and arabinoxylan modulate digestion processes and SCFA pool size in the large intestine and faecal microbial composition in pigs. *Br. J. Nutr.* **2014**, *112*, 1837–1849. [CrossRef]
8. Enriquez, A.B.; Ten Caten, F.; Ghneim, K.; Sekaly, R.P.; Sharma, A.A. Regulation of Immune Homeostasis, Inflammation, and HIV Persistence by the Microbiome, Short-Chain Fatty Acids, and Bile Acids. *Annu. Rev. Virol.* **2023**, *10*, 397–422. [CrossRef]
9. McGlone, E.R.; Bloom, S.R. Bile acids and the metabolic syndrome. *Ann. Clin. Biochem.* **2019**, *56*, 326–337. [CrossRef]
10. Liu, Y.; Huang, K.; Zhang, Y.; Cao, H.; Guan, X. Dietary polyphenols maintain homeostasis via regulating bile acid metabolism: A review of possible mechanisms. *Food Funct.* **2023**, *14*, 9486–9505. [CrossRef]
11. Bing, H.; Li, Y.L. The role of bile acid metabolism in the occurrence and development of NAFLD. *Front. Mol. Biosci.* **2022**, *9*, 1089359. [CrossRef] [PubMed]
12. Meadows, V.; Kennedy, L.; Kundu, D.; Alpini, G.; Francis, H. Bile Acid Receptor Therapeutics Effects on Chronic Liver Diseases. *Front. Med.* **2020**, *7*, 15. [CrossRef] [PubMed]
13. Qin, S.; Tian, J.; Zhao, Y.; Wang, L.; Wang, J.; Liu, S.; Meng, J.; Wang, F.; Liu, C.; Han, J.; et al. Gardenia extract protects against intrahepatic cholestasis by regulating bile acid enterohepatic circulation. *J. Ethnopharmacol.* **2023**, *319*, 117083. [CrossRef]
14. Yu, L.; Liu, Y.; Wang, S.; Zhang, Q.; Zhao, J.; Zhang, H.; Narbad, A.; Tian, F.; Zhai, Q.; Chen, W. Cholestasis: Exploring the triangular relationship of gut microbiota-bile acid-cholestasis and the potential probiotic strategies. *Gut Microbes* **2023**, *15*, 2181930. [CrossRef] [PubMed]
15. Xiang, J.; Zhang, Z.; Xie, H.; Zhang, C.; Bai, Y.; Cao, H.; Che, Q.; Guo, J.; Su, Z. Effect of different bile acids on the intestine through enterohepatic circulation based on FXR. *Gut Microbes* **2021**, *13*, 1949095. [CrossRef] [PubMed]
16. Clifford, B.L.; Sedgeman, L.R.; Williams, K.J.; Morand, P.; Cheng, A.; Jarrett, K.E.; Chan, A.P.; Brearley-Sholto, M.C.; Wahlström, A.; Ashby, J.W.; et al. FXR activation protects against NAFLD via bile-acid-dependent reductions in lipid absorption. *Cell Metab.* **2021**, *33*, 1671–1684.e1674. [CrossRef]
17. Islam, K.B.; Fukiya, S.; Hagio, M.; Fujii, N.; Ishizuka, S.; Ooka, T.; Ogura, Y.; Hayashi, T.; Yokota, A. Bile acid is a host factor that regulates the composition of the cecal microbiota in rats. *Gastroenterology* **2011**, *141*, 1773–1781. [CrossRef]
18. De Valdez, G.F.; Martos, G.; Taranto, M.P.; Lorca, G.L.; Oliver, G.; de Ruiz Holgado, A.P. Influence of bile on beta-galactosidase activity and cell viability of Lactobacillus reuteri when subjected to freeze-drying. *J. Dairy Sci.* **1997**, *80*, 1955–1958. [CrossRef]
19. Livak, K.J.; Schmittgen, T.D. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta C_T}$ Method. *Methods* **2001**, *25*, 402–408. [CrossRef]
20. Penders, J.; Vink, C.; Driessen, C.; London, N.; Thijs, C.; Stobberingh, E.E. Quantification of *Bifidobacterium* spp., *Escherichia coli* and *Clostridium difficile* in faecal samples of breast-fed and formula-fed infants by real-time PCR. *FEMS Microbiol. Lett.* **2005**, *243*, 141–147. [CrossRef]
21. Fan, Y.; Ju, T.; Bhardwaj, T.; Korver, D.R.; Willing, B.P. Week-Old Chicks with High Bacteroides Abundance Have Increased Short-Chain Fatty Acids and Reduced Markers of Gut Inflammation. *Microbiol. Spectr.* **2023**, *11*, e0361622. [CrossRef] [PubMed]
22. Rinttilä, T.; Kassinen, A.; Malinen, E.; Krogius, L.; Palva, A. Development of an extensive set of 16S rDNA-targeted primers for quantification of pathogenic and indigenous bacteria in faecal samples by real-time PCR. *J. Appl. Microbiol.* **2004**, *97*, 1166–1177. [CrossRef] [PubMed]
23. Ohashi, Y.; Fujisawa, T. Analysis of Clostridium cluster XI bacteria in human feces. *Biosci. Microbiota Food Health* **2019**, *38*, 65–68. [CrossRef] [PubMed]
24. Pereira, A.M.; Maia, M.R.G.; Pinna, C.; Biagi, G.; Matos, E.; Segundo, M.A.; Fonseca, A.J.M.; Cabrita, A.R.J. Effects of Zinc Source and Enzyme Addition on the Fecal Microbiota of Dogs. *Front. Microbiol.* **2021**, *12*, 688392. [CrossRef]
25. Liu, Y.S.; Zhang, Y.Y.; Li, J.L.; Wang, X.F.; Xing, T.; Zhu, X.D.; Zhang, L.; Gao, F. Growth performance, carcass traits and digestive function of broiler chickens fed diets with graded levels of corn resistant starch. *Br. Poult. Sci.* **2020**, *61*, 146–155. [CrossRef]
26. Camp, L.K.; Southern, L.L.; Bidner, T.D. Effect of carbohydrate source on growth performance, carcass traits, and meat quality of growing-finishing pigs. *J. Anim. Sci.* **2003**, *81*, 2488–2495. [CrossRef]
27. Heo, J.M.; Agyekum, A.K.; Yin, Y.L.; Rideout, T.C.; Nyachoti, C.M. Feeding a diet containing resistant potato starch influences gastrointestinal tract traits and growth performance of weaned pigs. *J. Anim. Sci.* **2014**, *92*, 3906–3913. [CrossRef]
28. Li, T.J.; Dai, Q.Z.; Yin, Y.L.; Zhang, J.; Huang, R.L.; Ruan, Z.; Deng, Z.; Xie, M. Dietary starch sources affect net portal appearance of amino acids and glucose in growing pigs. *Animal* **2008**, *2*, 723–729. [CrossRef]
29. Bergh, M.; Razdan, A.; Åman, P. Nutritional influence of broiler chicken diets based on covered normal, waxy and high amylose barleys with or without enzyme supplementation. *Anim. Feed. Sci. Technol.* **1999**, *78*, 215–226. [CrossRef]
30. Tian, S.; Chu, Q.; Ma, S.; Ma, H.; Song, H. Dietary Fiber and Its Potential Role in Obesity: A Focus on Modulating the Gut Microbiota. *J. Agric. Food Chem.* **2023**, *71*, 14853–14869. [CrossRef]

31. Da Dalt, L.; Moregola, A.; Svecla, M.; Pedretti, S.; Fantini, F.; Ronzio, M.; Uboldi, P.; Dolfini, D.; Donetti, E.; Baragetti, A.; et al. The inhibition of inner mitochondrial fusion in hepatocytes reduces NAFL and improves metabolic profile during obesity by modulating bile acid conjugation. *Cardiovasc. Res.* **2023**, cvad169. [CrossRef] [PubMed]
32. Zhu, W.; Zhou, Y.; Tsao, R.; Dong, H.; Zhang, H. Amelioratory Effect of Resistant Starch on Non-alcoholic Fatty Liver Disease via the Gut-Liver Axis. *Front. Nutr.* **2022**, *9*, 861854. [CrossRef] [PubMed]
33. Ha, A.W.; Han, G.J.; Kim, W.K. Effect of retrograded rice on weight control, gut function, and lipid concentrations in rats. *Nutr. Res. Pract.* **2012**, *6*, 16–20. [CrossRef] [PubMed]
34. Nichenametla, S.N.; Weidauer, L.A.; Wey, H.E.; Beare, T.M.; Specker, B.L.; Dey, M. Resistant starch type 4-enriched diet lowered blood cholesterols and improved body composition in a double blind controlled cross-over intervention. *Mol. Nutr. Food Res.* **2014**, *58*, 1365–1369. [CrossRef] [PubMed]
35. Ocvirk, S.; O'Keefe, S.J.D. Dietary fat, bile acid metabolism and colorectal cancer. *Semin. Cancer Biol.* **2021**, *73*, 347–355. [CrossRef]
36. Jia, W.; Wei, M.; Rajani, C.; Zheng, X. Targeting the alternative bile acid synthetic pathway for metabolic diseases. *Protein Cell* **2021**, *12*, 411–425. [CrossRef]
37. Wahlström, A.; Sayin, S.I.; Marschall, H.U.; Bäckhed, F. Intestinal Crosstalk between Bile Acids and Microbiota and Its Impact on Host Metabolism. *Cell Metab.* **2016**, *24*, 41–50. [CrossRef]
38. Fiorucci, S.; Baldoni, M.; Ricci, P.; Zampella, A.; Distrutti, E.; Biagioli, M. Bile acid-activated receptors and the regulation of macrophages function in metabolic disorders. *Curr. Opin. Pharmacol.* **2020**, *53*, 45–54. [CrossRef]
39. Adhikari, A.A.; Ramachandran, D.; Chaudhari, S.N.; Powell, C.E.; Li, W.; McCurry, M.D.; Banks, A.S.; Devlin, A.S. A Gut-Restricted Lithocholic Acid Analog as an Inhibitor of Gut Bacterial Bile Salt Hydrolases. *ACS Chem. Biol.* **2021**, *16*, 1401–1412. [CrossRef]
40. Katafuchi, T.; Makishima, M. Molecular Basis of Bile Acid-FXR-FGF15/19 Signaling Axis. *Int. J. Mol. Sci.* **2022**, *23*, 6046. [CrossRef]
41. Houten, S.M.; Watanabe, M.; Auwerx, J. Endocrine functions of bile acids. *EMBO J.* **2006**, *25*, 1419–1425. [CrossRef] [PubMed]
42. Zhang, B.; Kuipers, F.; de Boer, J.F.; Kuivenhoven, J.A. Modulation of Bile Acid Metabolism to Improve Plasma Lipid and Lipoprotein Profiles. *J. Clin. Med.* **2021**, *11*, 4. [CrossRef] [PubMed]
43. Goldstein, J.; Levy, C. Novel and emerging therapies for cholestatic liver diseases. *Liver Int.* **2018**, *38*, 1520–1535. [CrossRef] [PubMed]
44. Jia, W.; Xie, G.; Jia, W. Bile acid-microbiota crosstalk in gastrointestinal inflammation and carcinogenesis. *Nat. Rev. Gastroenterol. Hepatol.* **2018**, *15*, 111–128. [CrossRef]
45. Shih, D.M.; Kast-Woelbern, H.R.; Wong, J.; Xia, Y.R.; Edwards, P.A.; Lusis, A.J. A role for FXR and human FGF-19 in the repression of paraoxonase-1 gene expression by bile acids. *J. Lipid Res.* **2006**, *47*, 384–392. [CrossRef]
46. Devlin, A.S.; Fischbach, M.A. A biosynthetic pathway for a prominent class of microbiota-derived bile acids. *Nat. Chem. Biol.* **2015**, *11*, 685–690. [CrossRef]
47. Wang, Y.; Yu, Y.; Li, L.; Zheng, M.; Zhou, J.; Gong, H.; Feng, B.; Wang, X.; Meng, X.; Cui, Y.; et al. Bile acid-dependent transcription factors and chromatin accessibility determine regional heterogeneity of intestinal antimicrobial peptides. *Nat. Commun.* **2023**, *14*, 5093. [CrossRef]
48. Goldberg, A.A.; Beach, A.; Davies, G.F.; Harkness, T.A.; Leblanc, A.; Titorenko, V.I. Lithocholic bile acid selectively kills neuroblastoma cells, while sparing normal neuronal cells. *Oncotarget* **2011**, *2*, 761–782. [CrossRef]