agriculture

MDPI

Article

Dietary Serine Supplementation Improves Growth Performance, Intramuscular Fat Content, and Composition of Gut Microbes and Metabolites in Growing–Finishing Pigs

Yiting Guo [1,2], Fang He [1,2], Zhiying Deng [1,2], Jie Yin [1], Guiping Guan [3,*], Zhengjun Xie [4,*] and Xihong Zhou [2,5,*]

1 College of Animal Science and Technology, Hunan Agricultural University, Changsha 410128, China; 16673232280@163.com (F.H.); dengzhiying1002@163.com (Z.D.); yinjie@hunau.edu.cn (J.Y.)
2 Key Laboratory of Agro-Ecological Processes in Subtropical Region, Institute of Subtropical Agriculture, The Chinese Academy of Sciences, Changsha 410125, China
3 College of Bioscience & Biotechnology, Hunan Agricultural University, Changsha 410128, China
4 Shuangbaotai Group Co., Ltd., Nanchang 330095, China
5 College of Advanced Agricultural Sciences, University of Chinese Academy of Sciences, Beijing 100049, China
* Correspondence: guanguiping@hunau.edu.cn (G.G.); xiezj@sbtjt.com (Z.X.); xhzhou@isa.ac.cn (X.Z.)

Abstract: Serine is widely involved in antioxidant synthesis, immune response, and fat metabolism. However, it remains unclear whether dietary serine supplementation affects fat deposition in the skeletal muscles of pigs. Thus, we explored the effects of dietary serine supplementation on growth performance, meat quality, and composition of gut microbes and their metabolites in growing–finishing pigs. Forty-eight boars weighing approximately 20 kg were fed either a basal diet or a basal diet supplemented with 0.15% serine. The results showed that dietary serine increased the daily weight gain of pigs and improved serum antioxidant capacity as indicated by the decreased malondialdehyde content and increased glutathione and superoxide dismutase content. Pigs supplemented with serine had better meat quality, as shown by the lower drip loss and higher IMF content. Furthermore, dietary serine increased the relative abundance of *Streptococcus* and *Lactobacillus* and decreased the relative abundance of *Clostridium_sensu_stricto_1* and *Terrisporobacter*. Differential microbial metabolites were mostly enriched in metabolic pathways related to lipid synthesis, such as alpha-linolenic acid metabolism and steroid hormone biosynthesis. Correlation analysis showed that the altered metabolites were closely related to the intestinal microbiota. In conclusion, our results suggested that serine serves as a potential additive for improving IMF content in growing–finishing pigs.

Keywords: finishing pig; gut microbe; meat quality; metabolite; serine

Citation: Guo, Y.; He, F.; Deng, Z.; Yin, J.; Guan, G.; Xie, Z.; Zhou, X. Dietary Serine Supplementation Improves Growth Performance, Intramuscular Fat Content, and Composition of Gut Microbes and Metabolites in Growing–Finishing Pigs. *Agriculture* **2024**, *14*, 349. https://doi.org/10.3390/agriculture14030349

Academic Editor: Vito Laudadio

Received: 21 December 2023
Revised: 7 February 2024
Accepted: 12 February 2024
Published: 22 February 2024

1. Introduction

Markets have preferred pig breeds with fast growth rates and have neglected the importance of meat quality under intensive cultivation conditions for recent decades to meet the needs of the masses [1–3]. However, with economic development and the increasing focus on health, more attention has been paid to meat quality. Dietary nutrients can improve meat quality in pigs. For example, adding β-glucan in finishing pigs can increase intramuscular fat (IMF) content and adjust the ratio of saturated and unsaturated fatty acids [4]; fermented okara can improve meat color and glutathione peroxidase (GSH-PX) and total superoxide dismutase (T-SOD) activity, which are beneficial to meat quality [5]. Moreover, certain functional amino acids, including tryptophan, threonine, arginine, and leucine, improve meat quality [6]. Additionally, meat color and fatty acid composition improved and IMF content increased in growing–finishing pigs supplemented with arginine and glutamic acid [7].

Serine, also known as β-hydroxyalanine, is a non-essential amino acid belonging to the neutral aliphatic group containing hydroxyl amino acids. L-serine is mainly used

as a basic amino acid to form proteins and exists in many feedstuffs. Thus, serine has been commonly considered as nutritionally non-essential and it has not been applied in pig production. But in fact, serine participates in various metabolic pathways, including glutathione, purine, and pyrimidine synthesis [8]. It enhances the host antioxidant abilities by promoting the synthesis of GSH and folate-dependent NADPH [9]. Notably, several studies have investigated the application of serine as a feed additive in animal production. It is suggested that serine has the same growth-promoting effects as those demonstrated by glycine in broiler chickens [10]. Our previous study showed that the ratio of serine to glycine affected the IMF content in growing–finishing pigs fed a low-protein diet [11]. It has been demonstrated that both exogenous and endogenous serine residues inhibit lipid deposition in the liver [12]. However, whether dietary supplementation with serine alone affects fat deposition in the skeletal muscle of pigs remains unknown.

The gut microbiota ecosystem is critical for maintaining the proper nutritional, metabolic, and physiological functions of the pig. Notably, the gut microbiota could affect host lipid metabolism including lipid intake and deposition. The gut microbial profiles of obese Shaziling pigs was different from lean Yorkshire pigs [13]. Obese Jinhua pigs had a higher abundance of archaeal species in association with higher lipid accumulation [14]. Furthermore, transplantation of the microbiota derived from obese Ningxiang pigs could enhance the accumulation of fat in the skeletal muscle of lean Duroc × Landrace × Yorkshire pigs through regulating carnitine metabolism [15]. The intestinal microbiota composition has been reported to mediate the beneficial effects of dietary functional nutrients on meat quality. For instance, the byproduct of rice distilling improved meat drip loss by influencing the relative abundance of *Erysipelotricchaceae* and *Porphyromonadaceae* in growing–finishing pigs [16]. We previously found that serine increased the relative abundance of Clostridia and Firmicutes and the number of operational taxonomic units (OTUs) in mice [17], indicating its effects on microbiota composition. However, whether serine affects meat quality by modulating intestinal microbiota composition in growing–finishing pigs remains unknown. In this study, we aimed to explore the effects of serine on the growth performance, meat quality, and serum biochemical parameters of growing–finishing pigs. Furthermore, we determined the composition of intestinal microbes and their metabolites to investigate whether microbes were involved in the effects of serine supplementation on growing–finishing pigs.

2. Materials and Methods

2.1. Experimental Design

In this study, 48 healthy crossbred (Duroc × Landrace × Yorkshire) male pigs with an average initial body weight (BW) of approximately 20 kg were selected. Pigs were randomly divided into two groups (six pens per treatment and four pigs per pen) and fed either a basal diet (CONT) or a basal diet supplemented with 0.15% serine (SER). Serine was purchased from Zhangjiagang SpecomBiochemical Co., LTD (Suzhou, China). Serine was used as a feed additive and firstly mixed into the premix and then mixed with the base diet and stirred well. The animals had ad libitum access to water and feed during the 17-week experimental period. The initial and final BW and dietary consumption of each pen were recorded during the experiment. The average daily feed intake (ADFI), average daily gain (ADG), and feed-to-gain (F/G) ratio were calculated. All nutrients conformed to the requirements of the National Research Council (NRC) (2012), and the diet composition and nutrient level are shown in Table 1. Specifically, the control pigs received diets with 17.51%, 15.57%, and 12.55% protein in the three growth stages, while the pigs supplemented with serine received diets with 17.66%, 15.72%, and 12.7% protein, respectively. All pigs in the two groups received diets with the same level of digestible energy, minerals, and vitamins. The experimental protocol was approved by the Protocol Management and Review Committee of the Institute of Subtropical Agriculture, Chinese Academy of Sciences.

Table 1. Basal diet composition and nutrient level.

Item	20~30 kg	30~60 kg	60~120 kg
Corn, %	65.00	72.30	59.00
Wheat bran, %	6.00	3.00	/
Rice, %	/	/	20.00
Soybean meal [#], %	25.00	19.50	14.00
Soybean oil, %	/	1.20	3.00
Limestone, %	1.00	0.80	0.80
calcium hydrogen phosphate, %	0.80	0.70	0.50
NaCl, %	0.40	0.45	0.30
L-lysine hydrochloride, %	0.30	0.60	0.25
DL-methionine, %	0.10	0.10	/
L-threonine, %	0.20	0.20	/
L-tryptophan, %	/	0.03	/
Zeolite powder	0.08	/	1.03
Premix *, %	1.12	1.12	1.12
Total, %	100	100	100
Calculated nutrient level			
DE (MJ/kg)	13.86	14.28	14.03
CP, %	17.51	15.57	12.55
Ca, %	0.64	0.53	0.46
Total P, %	0.50	0.43	0.38
P, %	0.23	0.20	0.17
Lysine, %	1.03	1.12	0.71
Methionine, %	0.36	0.33	0.2
Threonine, %	0.73	0.65	0.37
Tryptophan, %	0.15	0.15	0.10

CP = crude protein; DE = digestible energy. [#] Soybean meal contains 43% protein. * Provided for per kilogram of diet: vitamin A, 13,000 IU; vitamin D_3, 4000 IU; vitamin E, 32 IU; vitamin K, 4 mg; vitamin B_1, 4 mg; vitamin B_2, 10 mg; vitamin B_6, 6 mg; vitamin B_{12}, 6 mg; vitamin B_3, 48 mg; vitamin B_5, 24 mg; folic acid, 2 mg; biotin, 0.2 mg; $FeSO_4$, 180 mg; $CuSO_4$, 12 mg; $ZnSO_4$, 140 mg; $MnSO_4$, 8 mg; $Ca(IO_3)_2$, 0.4 mg; Na_2SeO_3, 0.2 mg.

2.2. Sample Collection

Fresh fecal samples were collected from all the pigs the day before slaughter. All fecal samples were transferred into sterile microcentrifuge tubes immediately and placed in liquid nitrogen, then stored at −80 °C for further microbial and metabolomic analysis [18]. Blood samples were collected through anterior vena cava puncture before slaughter and centrifuged at 3000× *g* at 4 °C for 10 min to collect serum samples. All serum samples were stored at −80 °C until analysis. *Longissimus dorsi* muscle samples between the 6th and 7th rib were obtained and then stored at 4 °C for 24 h for meat quality analysis.

2.3. Biochemical Parameters

The serum samples were thawed from −80 °C, and centrifuged at 3000× *g* at 4 °C for 10 min after thawing completely. The supernatant was taken to detect antioxidant parameters. Commercially available kits (Jiancheng, Nanjing, China) were used to measure malondialdehyde (MDA) content, and kits (BYabscience, Nanjing, China) were used to detect glutathione (GSH) and superoxide dismutase (SOD) content.

2.4. Meat Quality

Initial and ultimate pH values ($pH_{45\ min}$ and $pH_{24\ h}$) post mortem were measured and meat color was evaluated on a freshly cut surface of *longissimus dorsi* using a colorimeter with the parameters L* (brightness), a* (redness), and b* (yellowness) [19]. *Longissimus dorsi* samples were weighed before and after 24 h storage at 4 °C (W1 and W2, respectively), and drip loss was calculated as ((W1 − W2)/W1) [20]. Intramuscular fat was defined as the ratio of crude fat weight to the *longissimus dorsi* muscle weight. Crude fat was extracted using Soxhlet extraction.

2.5. Fecal Microbiota Profiling

Fecal DNA was obtained using a Magnetic Soil and Stool DNA Kit (TianGen, Shanghai, China; Catalog #: DP712). Specific primers were selected for the V3–V4 region of 16S rRNA. Phusion High-Fidelity PCR Master Mix with GC Buffer was provided by New England Biolabs Company (Ipswich, MA, USA) for PCR. Amplicons were purified using a Universal DNA Purification Kit (TianGen, Shanghai, China, Catalog #: DP214) and sequenced on Illumina Novaseq6000 platforms. Fastp (version 0.23.1) software was used for quality filtering to acquire high-quality clean tags. Microbial sequences were categorized into OTUs based on 97% identity. Species annotations for each representative sequence were used to obtain species messages, including microbial relative abundance and distribution, evenness messages, and mutual or specific OTUs. Intestinal microbial alpha and beta diversity were analyzed using QIIME2 software (Version QIIME2-202006). Moreover, microbial community differences were directly displayed in dimensionality reduction maps, including principal coordinate analysis (PCoA) through multi-sequence comparison of OTUs.

2.6. Determination of Fecal Metabolites

Mixtures of fecal samples and an 80% methanol solution were centrifuged to obtain the supernatant, and metabolites were analyzed using liquid chromatography–mass spectrometry (LC-MS). Metabolites were detected using a high-resolution series mass spectrometer and annotated after matching the exact molecular mass data (m/z) based on the online Human Metabolome Database (HMDB). Differential metabolites were detected using Student's t-test. Multiple tests were implemented using false discovery rate (FDR) to assess significantly altered metabolites in accordance with the p-value. The acquired data were imported for SIMCA Statistical Analysis and principal component analysis (PCA). We performed a correlation analysis based on the Pearson correlation coefficient after obtaining microbial and metabolomic data to explore the relative extent of metabolite and microbial species diversity.

2.7. Statistical Analysis

All data were analyzed using one-way ANOVA followed by Duncan's multiple comparison test. Data statistics software (SPSS 25.0) was used, and a probability value (p-value) < 0.05 was set as a statistically significant difference.

3. Results

3.1. Growth Performance and Serum Biochemical Parameters

The growth performance of the pigs is shown in Table 2. Compared to control pigs, pigs in the SER group had higher final BW and ADFI and lower F/G; however, the differences were not significant. ADG was significantly higher in pigs in the SER group than in those in the CONT group. As shown in Table 3, serine significantly decreased the MDA content and increased the GSH and SOD contents in the serum of growing–finishing pigs ($p < 0.05$).

Table 2. Growth performance of growing–finishing pigs.

	CONT	SER
Initial BW, kg	20.95 ± 0.76	20.35 ± 0.38
Final BW, kg	116.8 ± 7.1	125.2 ± 4.9
ADG, kg	0.806 ± 0.059 [a]	0.871 ± 0.026 [b]
ADFI, kg	2.25 ± 0.16	2.29 ± 0.04
F/G	2.80 ± 0.05	2.63 ± 0.10

[a,b] indicates a significant difference between the treatment groups ($p < 0.05$), $n = 6$. Data are shown as mean ± SD. CONT, pigs fed a basal diet. SER, pigs fed a basal diet supplemented with 0.15% serine. Initial BW, initial body weight; final BW, final body weight; ADG, average daily gain; ADFI, average daily feed intake; F/G, the ratio of feed to gain.

Table 3. Serum biochemical parameters of growing–finishing pigs.

	CONT	SER
GSH, ng/mL	129.4 ± 17.4 [a]	196.9 ± 19.1 [b]
SOD, ng/mL	197.6 ± 29.3 [a]	286.4 ± 17.2 [b]
MDA, nmol/mL	4.06 ± 0.40 [a]	1.91 ± 0.24 [b]

[a,b] indicates a significant difference between the treatment groups ($p < 0.05$), $n = 6$. Data are shown as mean ± SD. CONT, pigs fed a basal diet. SER, pigs fed a basal diet supplemented with 0.15% serine. GSH, glutathione; SOD, superoxide dismutase; MDA, malondialdehyde.

3.2. Meat Quality

As shown in Table 4, we did not observe significant differences in meat color (L, a, and b), $pH_{45\ min}$, or $pH_{24\ h}$ value ($p > 0.05$) between the treatment groups. However, drip loss was significantly lower ($p < 0.05$) and IMF content was significantly higher ($p < 0.05$) in pigs supplemented with 0.15% serine than in the control pigs.

Table 4. Meat quality traits of the growing–finishing pigs.

	CONT	SER
Color		
L	45.23 ± 1.56	44.11 ± 1.28
a	13.86 ± 0.33	14.48 ± 0.48
b	5.40 ± 0.57	5.08 ± 0.46
pH45 min	6.60 ± 0.09	6.42 ± 0.11
pH24 h	5.60 ± 0.07	5.53 ± 0.07
Drip loss	3.58 ± 0.50 [a]	2.24 ± 0.33 [b]
Intramuscular fat	2.26 ± 0.18 [a]	2.94 ± 0.17 [b]

[a,b] indicates a significant difference between the treatment groups ($p < 0.05$), $n = 6$. Data are shown as mean ± SD. CONT, pigs fed a basal diet. SER, pigs fed a basal diet supplemented with 0.15% serine.

3.3. Fecal Microbiota Composition

The fecal microbiota composition was compared using a 16S rDNA phylogenetic approach. The Venn diagram indicated that pigs in the CONT group had 1827 unique OTUs, whereas pigs in the SER group had 2062 unique OTUs and 1489 universal OTUs (Figure 1A). Fecal microbial α-diversity had no significant difference between different groups, as indicated using Chao1 index (Figure 1B). The major microbes at the class level included Clostridia, Bacilli, Bacteroidia, Gammaproteobacteria, and Spirochaetia, and serine increased the relative abundance of Bacilli and Bacteroidia and decreased the relative abundance of Clostridia (Figure 1C). The major microbes at the genus level included *Streptococcus*, *Clostridium_sensu_stricto_1*, *Lactobacillus*, and *Terrisporobacter*. Serine supplementation increased the relative abundance of *Streptococcus* and *Lactobacillus*, and decreased the relative abundance of *Clostridium_sensu_stricto_1* and *Terrisporobacter* (Figure 1D).

As indicated by the results of the weighted UniFrac *t*-test and PCoA (Figure 2A) based on the Jaccard distance matrix (Figure 2B), the beta diversity was distinctively different between pigs in the CONT and SER groups. We then filtered out the significantly different microbes at the genus and species levels using a statistical *t*-test. The results showed that at the genus level, the relative abundances of *Lactobacillus*, *Prevotellaceae_UCG-001*, and *Phascolarctobacterium* were significantly increased, whereas the relative abundances of *Clostridium_sensu_stricto-1*, *Terrisporobacter*, *Eubacterium_coprostanollgenes group*, *Listeria*, *Candidatus_Soleaferrea*, and *UCG-009* were significantly decreased by serine supplementation (Figure 2C). At the species level, the relative abundance of *Clostridium butyricum* was significantly decreased, and that of *Lactobacillus delbrueckii* was significantly increased by serine supplementation (Figure 2D).

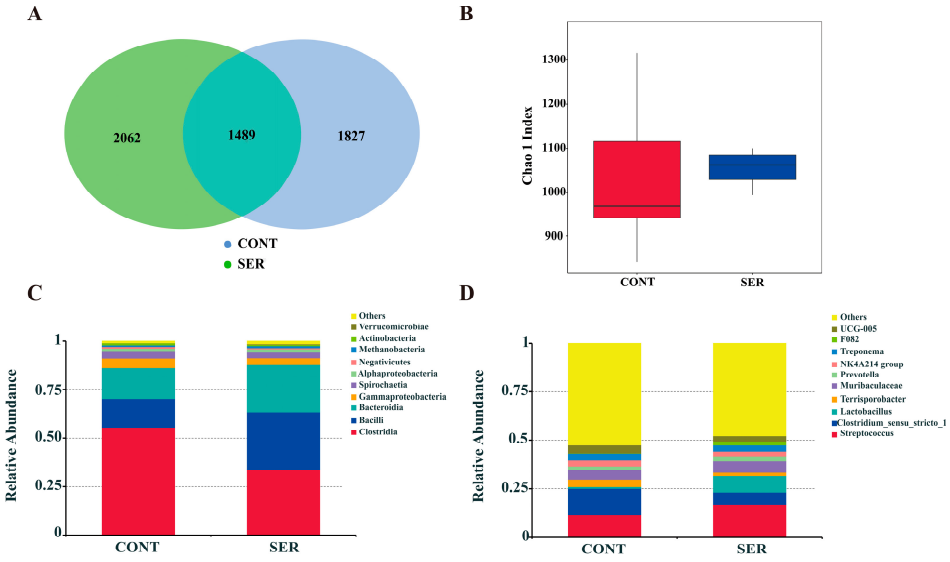

Figure 1. Bacteria community variety and richness: (**A**) Venn diagram. (**B**) Chao1 index. Relative abundance of top 10 microbiota community at class level (**C**) and genus level (**D**). CONT, pigs fed a basal diet. SER, pigs fed a basal diet supplemented with 0.15% serine.

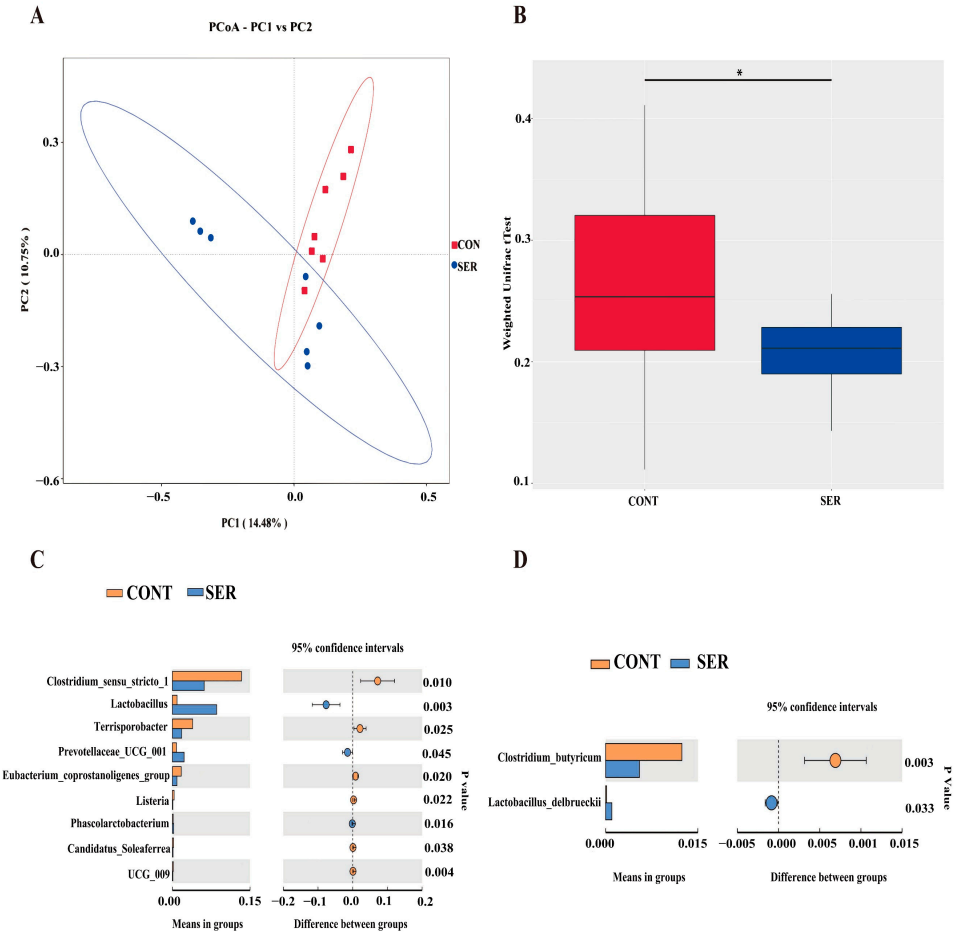

Figure 2. Microbial β-diversity and different microbes between groups based on *t*-test analysis: Principal coordinate analysis (PCoA) based on the Jaccard distance matrix (**A**). Box plot, displayed by weighted Unifrac *t*-test (**B**). * $p < 0.05$. Different microbes between groups at genus (**C**) and species (**D**) level, analyzed by *t*-test. CONT, pigs fed a basal diet. SER, pigs fed a basal diet supplemented with 0.15% serine.

3.4. Fecal Metabolites

Untargeted metabolomics was used to explore differences in fecal metabolite composition. The data were processed using partial least squares discriminant analysis (PLS-DA), visually demonstrating an obvious discrepancy between pigs in the CONT and SER groups (Figure 3A,B). The threshold was constructed as VIP > 1.0, fold change (FC) > 1.2, or FC < 0.833 and $p < 0.05$ to obtain the differentially expressed metabolites (Figure 3C,D). For the positive ion, significant differences were detected in 24 metabolites, with 20 metabolites increased and 4 metabolites decreased. For the negative ion, remarkable differences were observed in 13 metabolites, with 10 increased and 3 decreased.

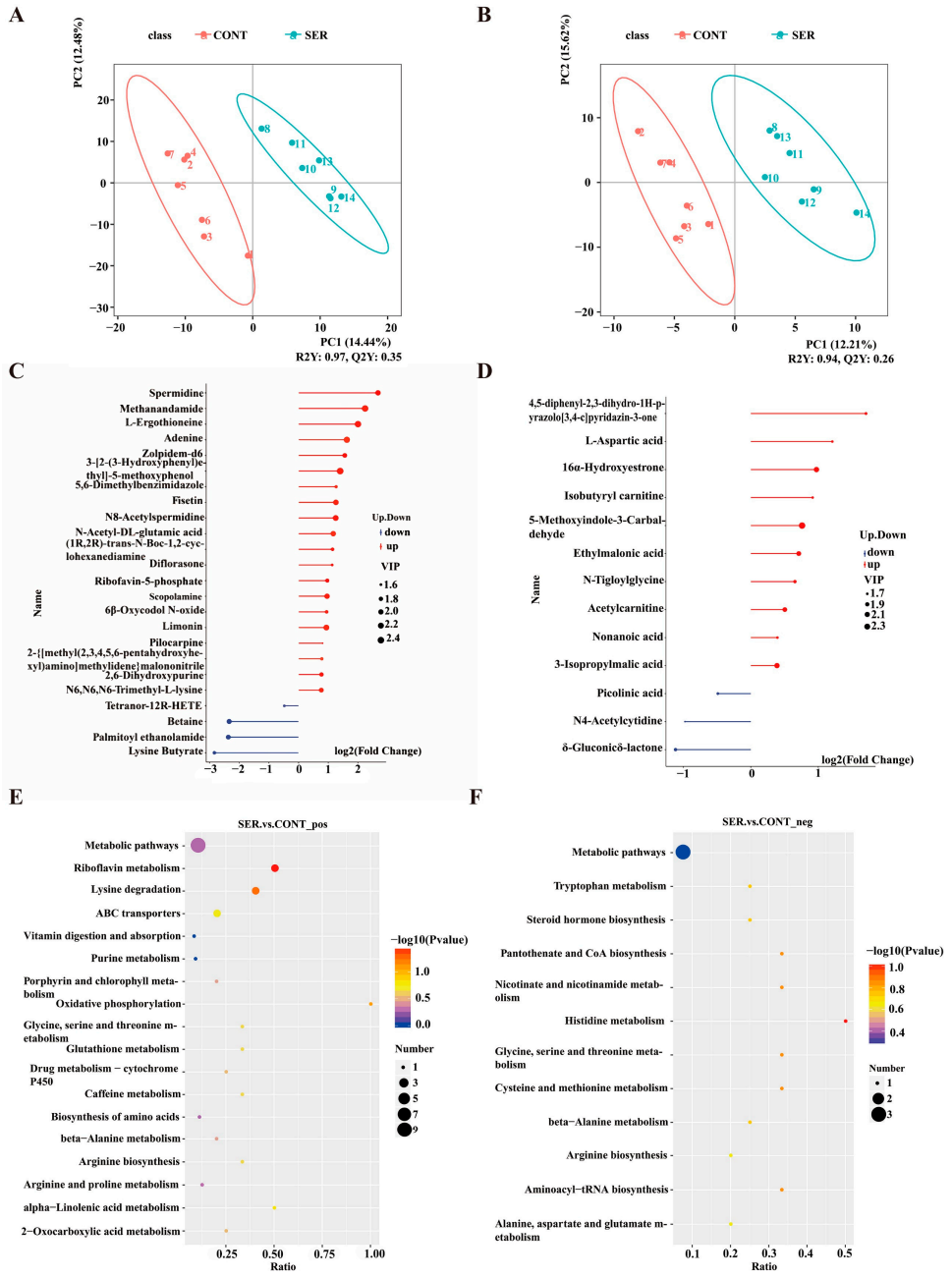

Figure 3. The composition of fecal metabolites. Partial least-square discriminant analysis (PLS-DA) of positive (**A**) and negative (**B**) ion modes. Stem graph of differential metabolites in the positive (**C**) and negative (**D**) ion modes. KEGG pathway analysis of metabolism in positive (**E**) and negative (**F**) ion modes between treatment groups. CONT, pigs fed a basal diet. SER, pigs fed a basal diet supplemented with 0.15% serine.

KEGG pathway annotation and enrichment analysis revealed the pathways affected by serine supplementation. As shown in the KEGG enrichment map, apparent differences were observed between the CONT and SER groups (Figure 3E,F). In the positive ion mode, differential metabolites were mainly enriched in riboflavin metabolism; lysine degradation; oxidative phosphorylation; glycine, serine, and threonine metabolism; glutathione metabolism; amino acid biosynthesis; arginine biosynthesis; and alpha-linolenic acid metabolism. In the negative ion mode, differential metabolism was mainly enriched in tryptophan metabolism; glycine, serine, and threonine metabolism; steroid hormone biosynthesis; pantothenate and CoA biosynthesis; alanine, aspartate, and glutamate metabolism; histidine metabolism; arginine biosynthesis; cysteine and methionine metabolism; and aminoacyl-tRNA biosynthesis.

3.5. Correlation between Microbiota and Metabolites

Pearson's correlation analysis was performed to explore the correlation between the top five differentially expressed fecal microbes and the top ten differentially expressed fecal metabolites between the CONT and SER groups. The correlation Sankey diagram analysis clearly indicated a relationship between the intestinal microbiota and metabolites (Figure 4A,B). As shown in Figure 4C, there was a significant positive correlation between *Clostridium_sensu_stricto_1* and its metabolites (in the positive ion mode) including N8-Acetylspermidine, limonin, 6-phenyl-1,2,3,4-tetrahydro-2,5-benzodiazocin-1-one, 7-Methylxanthine, L-Ergothioneine, and spermidine ($p < 0.05$). As shown in Figure 4D, there was a significant positive correlation between *Anaerorhabdus_furcosa_group* and metabolites (in the negative ion mode) including16-Hydroxyestrone, 5-Methoxyindole-3-Carbaldehyde, Ethylmalonic acid, and L-aspartic acid ($p < 0.05$). *Clostridium_sensu_stricto_1* and UCG-009 were also positively correlated with certain differential metabolites, including 6-Hydroxyestrone and 3-Isopropylmalic acid, respectively ($p < 0.05$). *Phascolarctobacterium* and *Lactobacillus* were negatively correlated with the differential metabolites, as shown in Figure 4C,D ($p < 0.05$).

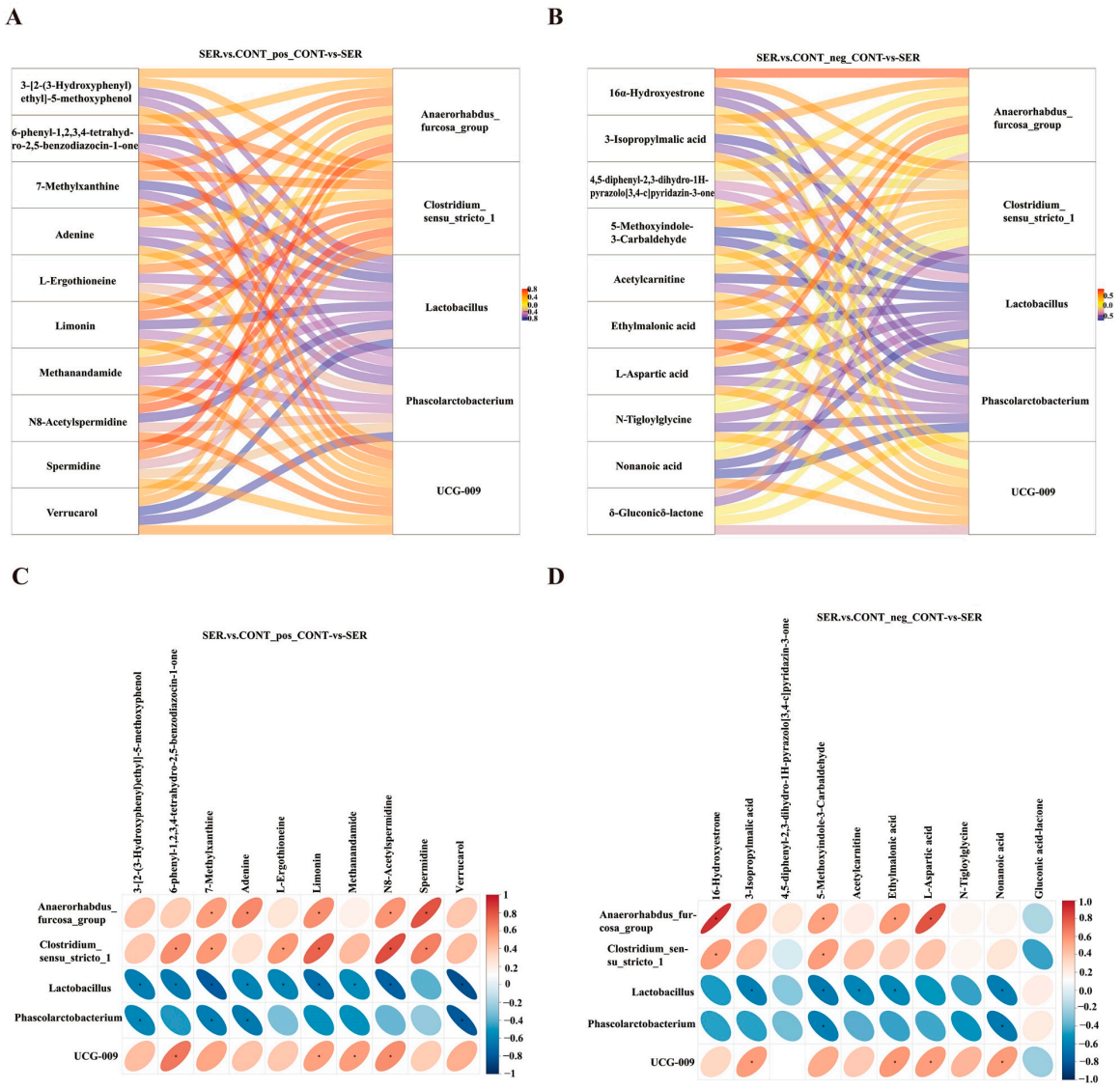

Figure 4. Correlations between intestinal microbiota and metabolites. Sankey diagram of differential intestinal microbiota in the positive (**A**) and negative (**B**) ion modes. The correlation heat map of differential intestinal microbiota and metabolites in the positive (**C**) and negative (**D**) modes. * $p < 0.05$.

4. Discussion

Serine is beneficial for anti-inflammatory action and antioxidation and can influence the composition of intestinal microbes, lipid metabolism, and protection of the intestinal mucosa. In this study, 0.15% serine was added to the diet of finishing pigs to explore its beneficial effects. Serine increased growth performance; promoted meat quality, characterized by higher IMF content and less drip loss; enhanced serum antioxidant capability; and altered the composition of intestinal microbes and metabolites in growing–finishing pigs. Correlation analysis indicated a significant correlation between *Clostridium_sensu_stricto_1* and *Lactobacillus* and certain lipid metabolites. These results indicated that serine might increase growth performance and meat quality by influencing intestinal microbes and their metabolism in growing–finishing pigs.

Recently, increasing attention has been paid to meat quality because of economic development and the increasing focus on health. Meat quality is commonly determined by marbling scores, IMF, pH values, color scores, moisture content, and drip loss. Among these characteristics, IMF and drip loss are two critical indices for evaluating meat quality. We

previously found that pigs had higher IMF content when a suitable ratio of serine to glycine was maintained in their diet [11]. In this study, dietary supplementation with serine alone increased IMF content, further suggesting that serine may positively affect IMF deposition. Previous studies have demonstrated the role of serine in lipid metabolism [21], and serine deficiency increases fat deposition in the liver [12]. Serine participates critically in one-carbon metabolism and promotes NADPH synthesis, which is involved in the regulation of lipid metabolism [22]. We previously found that serine activates mTOR signaling, which promotes lipogenesis [23].

Increased antioxidant capability can improve the integrity of the cell membrane, which helps cells hold water [24]. Serine can significantly enhance antioxidant capability by acting as an indirect precursor of GSH to promote its synthesis [25]. In this study, we further confirmed that serine supplementation increased serum GSH content in growing–finishing pigs. Moreover, the content of other antioxidant enzymes, such as SOD, also increased, and the content of MDA, a biomarker of oxidative stress [26], decreased, illustrating that pigs supplemented with serine had better antioxidative capability. These pigs also exhibited lower meat drip loss. Thus, serine may decrease drip loss via its strong ability to maintain the oxidative balance.

Gut microbes harbored in the gastrointestinal tract have been demonstrated to regulate host lipid metabolism in the intestine and other organs. A previous study screened the correlation between gut microbial profiles and carcass lean yield in a large number of Duroc pigs and found that those pigs with higher abundance of *Prevotella copri* had higher lipid percentages and more metabolic features prone to obesity [27]. Additionally, another study isolated a strain of *Lactobacillus reuteri* from obese Ningxiang pigs and found that this strain could promote the accumulation of fatty acid in the skeletal muscle of pigs [15]. It is suggested that gut microbes affect lipid metabolism mainly through their metabolites. The supernatant from a *Desulfovibrio* species culture medium was proved to enhance fat intake and deposition in intestinal epithelia cells [28]. L-carnitine and its derivatives, which could be affected by the Ningxiang pig-derived microbiota, was reported to increase fatty acid content in the skeletal muscle of pigs [15]. Indeed, a variety of dynamic microbiota exist in the enteric canal and are mainly associated with meat quality by regulating lipid metabolism [15]. A previous study showed that serine alters the alpha and beta diversity of gut microbes [17]. Similarly, in this study, serine supplementation altered the beta diversity of the intestinal microbiota in growing–finishing pigs, which indicated that serine could modulate the composition of gut microbes. Moreover, our results showed that Streptococcus was the predominant bacterial genus in growing–finishing pigs. The relative abundances of Lactobacillus and Streptococcus increased, whereas the relative abundances of *Terrisporobacter* and *Clostridium_sensu_stricto_1* were decreased in the intestines of growing–finishing pigs supplemented with serine. Notably, the relative abundances of *Lactobacillus* and *Prevotellaceae UCG-009*, which are positively correlated with IMF content [29], were increased by dietary supplementation with serine. Therefore, the alteration of gut microbes caused by serine supplementation may also be one of the reasons for the increased IMF content.

Significant differences were observed in the metabolites among pigs in different groups according to the metabolomic results. The metabolites that differed between the two groups were mainly lipids and lipid-like molecules, including acetyl-carnitine, ethylmalonic acid, nonanoic acid, organic acids, and derivatives, including N8-acetylspermidine and L-aspartic acid. Among these differential metabolites, acetylcarnitine can promote fat synthesis by providing acetyl groups to several substances, including acetyl-CoA [15]. Ethylmalonic acid participates in fatty acid synthesis by acting as a cofactor for ethylmalonyl-CoA decarboxylase [30]. The level of l-aspartic acid, which is increased by serine supplementation, positively correlates with the IMF content [31,32]. Moreover, the differentially expressed metabolites were mostly enriched in metabolic pathways related to lipid biosynthesis, including alpha-linolenic acid metabolism, tryptophan metabolism [33,34], and steroid hormone biosynthesis. It is not only intestinal microbes that affect IMF content but

also their metabolites [35]. Thus, enhanced metabolite content and metabolic pathways associated with lipid metabolism may be other factors promoting IMF accumulation in pigs supplemented with serine. Importantly, the correlation analysis between microbes and metabolites indicated that the alteration of metabolites might be related to changes in the intestinal microbiota, as microbes, including *Anaerorhabdus_furcosa_group*, *Clostridium_sensu stricto_1*, *Lactobacillus*, *Phascolarctobacterium*, and *UCG-009*, were significantly altered, and most of them were strongly linked with metabolites associated with lipid metabolism. Nevertheless, further studies are needed to explore whether serine directly or indirectly influences meat quality by modulating the intestinal composition of microbes and their metabolites.

Although we found that dietary serine increased IMF content in the *longissimus dorsi* muscle and affected microbiota composition and its metabolites, we did not provide direct evidence that these altered compositions of the microbiota and metabolites mainly contributed to the higher IMF content in the growing–finishing pigs. This is the limitation of this study. Many studies have reported the beneficial effects of gut microbes on meat quality in pigs. For example, short-chain fatty acids produced by gut microbial fermentation can directly affect the expression of genes related to lipid metabolism, and indirectly act as signaling molecules to regulate the accumulation of IMF [36]. Intestinal microorganisms could also regulate the expression of the gene encoding lipoprotein lipase by modulating the expression of angiopoietin-like 4, thus promoting the storage of triglycerides in adipocytes, which contributes to the increase in IMF content [37,38]. Additionally, the gut microbiota could inhibit the expression of adenosine monophosphate-activated protein kinase and promote the expression of fatty acid synthase, thereby improving fat storage in skeletal muscle. However, none of these studies explored the indispensable role of the gut microbiota in the accumulation of IMF or elucidated the related mechanism. Therefore, future studies should focus on whether dietary serine could still enhance IMF content in the *longissimus dorsi* muscle of antibiotic-treated and germ-free pigs, to elucidate the critical role of gut microbes that mediate the beneficial effects of serine on lipid deposition in skeletal muscle. Moreover, since gut bacteria cannot penetrate the gut barrier and reach skeletal muscle in healthy pigs, alterations in related metabolites in serum and skeletal muscle need to be determined to explore whether certain metabolites are responsible for the alteration of lipid metabolism in muscles. Additionally, a previous study showed that gut microbes could alter the intestinal expression of miRNAs as well as bacteria-derived microvesicles [39,40], which might reach the skeletal muscle via circulation and affect the expression of their target genes related to lipid accumulation.

5. Conclusions

Our results suggested that a diet supplemented with serine significantly improved growth performance and serum antioxidant ability of growing–finishing pigs. Importantly, dietary serine could partly improve meat quality, characterized by higher IMF content and lower drip loss. Furthermore, dietary serine improved the composition of microbiota and metabolites in the growing–finishing pigs. All these results indicated that serine could be used as an alternative promising additive for the feed of growing–finishing pigs.

Author Contributions: Conceptualization, X.Z. and J.Y.; methodology, X.Z.; software, F.H.; validation, Y.G., F.H. and Z.D.; formal analysis, Z.D.; investigation, F.H.; resources, Z.X.; data curation, Y.G.; writing—original draft preparation, Y.G.; writing—review and editing, X.Z.; visualization, Y.G.; supervision, J.Y. and X.Z.; project administration, X.Z.; funding acquisition, G.G. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the Hunan Provincial Science and Technology Department (2021JJ30320), Hunan Provincial Department of Education(23A0186), the Science and Technology Innovation Program of Hunan Province (2023RC1074), and the Youth Innovation Promotion Association CAS and China Agriculture Research System of MOF and MARA (CARS-35).

Institutional Review Board Statement: This study was conducted according to the principles of the animal welfare committee of the Institute of Subtropical Agriculture, Chinese Academy of Sciences, and was approved by the animal welfare committee of the Institute of Subtropical Agriculture, Chinese Academy of Sciences.

Informed Consent Statement: Informed consent was obtained from all individual participants included in the study.

Data Availability Statement: The 16S rDNA gene sequence data have been deposited in the NCBI Bioproject database (URL: https://www.ncbi.nlm.nih.gov/bioproject/) (accessed on 25 December 2023), registration number PRJNA994863.

Conflicts of Interest: Author Zhengjun Xie was employed by the Shuangbaotai Group Co., Ltd. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

1. Liu, B.; Chen, Y.; Li, Q.; Zhong, Z.; Tan, Y.; Zhang, S.; Zhu, L. Effects of Breed and Gender Effect on Pork Quality Traits. *Southwest. China J. Agric. Sci.* **2019**, *32*, 2222–2225.
2. Wang, Y.; Thakali, K.; Morse, P.; Shelby, S.; Chen, J.; Apple, J.; Huang, Y. Comparison of Growth Performance and Meat Quality Traits of Commercial Cross-Bred Pigs versus the Large Black Pig Breed. *Animals* **2021**, *11*, 200. [CrossRef] [PubMed]
3. Chen, B.L.; Li, D.Y.; Leng, D.; Kui, H.; Bai, X.; Wang, T. Gut microbiota and meat quality. *Front. Microbiol.* **2022**, *13*, 951726. [CrossRef] [PubMed]
4. Luo, J.; Zeng, D.; Cheng, L.; Mao, X.; Yu, J.; Yu, B.; Chen, D. Dietary beta-glucan supplementation improves growth performance, carcass traits and meat quality of finishing pigs. *Anim. Nutr.* **2019**, *5*, 380–385. [CrossRef]
5. Tian, Z.; Deng, D.; Cui, Y.; Chen, W.; Yu, M.; Ma, X. Diet supplemented with fermented okara improved growth performance, meat quality, and amino acid profiles in growing pigs. *Food Sci. Nutr.* **2020**, *8*, 5650–5659. [CrossRef]
6. Ma, X.; Yu, M.; Liu, Z.; Deng, D.; Cui, Y.; Tian, Z.; Wang, G. Effect of amino acids and their derivatives on meat quality of finishing pigs. *J. Food Sci. Technol.* **2020**, *57*, 404–412. [CrossRef]
7. Hu, C.J.; Jiang, Q.Y.; Zhang, T.; Yin, Y.L.; Li, F.N.; Deng, J.P.; Wu, G.Y.; Kong, X.F. Dietary supplementation with arginine and glutamic acid modifies growth performance, carcass traits, and meat quality in growing-finishing pigs. *J. Anim. Sci.* **2017**, *95*, 2680–2689. [CrossRef] [PubMed]
8. He, L.; Long, J.; Zhou, X.; Liu, Y.; Li, T.; Wu, X. Serine is required for the maintenance of redox balance and proliferation in the intestine under oxidative stress. *FASEB J.* **2020**, *34*, 4702–4717. [CrossRef]
9. Lionaki, E.; Gkikas, I.; Daskalaki, I.; Ioannidi, M.-K.; Klapa, M.I.; Tavernarakis, N. Mitochondrial protein import determines lifespan through metabolic reprogramming and de novo serine biosynthesis. *Nat. Commun.* **2022**, *13*, 651. [CrossRef]
10. Waterhouse, H.N.; Scott, H.M. Effect of Different Proteins and Protein Levels on the Glycine Need of the Chick Fed Purified Diets. *Poult. Sci.* **1961**, *40*, 1160–1165. [CrossRef]
11. Zhou, X.; Liu, Y.; Zhang, L.; Kong, X.; Li, F. Serine-to-glycine ratios in low-protein diets regulate intramuscular fat by affecting lipid metabolism and myofiber type transition in the skeletal muscle of growing-finishing pigs. *Anim. Nutr.* **2021**, *7*, 384–392. [CrossRef]
12. He, L.; Liu, Y.; Liu, D.; Feng, Y.; Yin, J.; Zhou, X. Exogenous and Endogenous Serine Deficiency Exacerbates Hepatic Lipid Accumulation. *Oxid. Med. Cell. Longev.* **2021**, *2021*, 4232704. [CrossRef]
13. Ma, J.; Duan, Y.; Li, R.; Liang, X.; Li, T.; Huang, X.; Yin, Y.; Yin, J. Gut microbial profiles and the role in lipid metabolism in Shaziling pigs. *Anim. Nutr.* **2022**, *9*, 345–356. [CrossRef]
14. Zhao, G.; Xiang, Y.; Wang, X.; Dai, B.; Zhang, X.; Ma, L.; Yang, H.; Lyu, W.; Jia, Z. Exploring the Possible Link between the Gut Microbiome and Fat Deposition in Pigs. *Oxidative Med. Cell. Longev.* **2022**, *2022*, 1098892. [CrossRef] [PubMed]
15. Yin, J.; Li, Y.; Tian, Y.; Zhou, F.; Ma, J.; Xia, S.; Yang, T.; Ma, L.; Zeng, Q.; Liu, G.; et al. Obese Ningxiang pig-derived microbiota rewires carnitine metabolism to promote muscle fatty acid deposition in lean DLY pigs. *Innovation* **2023**, *4*, 100486. [CrossRef] [PubMed]
16. Nguyen Cong, O.; Bernard, T.; Pham Kim, D.; Do Duc, L.; Nassim, M.; Nguyen Thi, H.; Nguyen Hoang, T.; Georges, D.; Jerome, B.; Vu Dinh, T.; et al. Growth performance, carcass quality characteristics and colonic microbiota profiles in finishing pigs fed diets with different inclusion levels of rice distillers' by-product. *Anim. Sci. J. Nihon Chikusan Gakkaiho* **2019**, *90*, 948–960. [CrossRef] [PubMed]
17. Zhang, H.; Hua, R.; Zhang, B.; Zhang, X.; Yang, H.; Zhou, X. Serine Alleviates Dextran Sulfate Sodium-Induced Colitis and Regulates the Gut Microbiota in Mice. *Front. Microbiol.* **2018**, *9*, 3062. [CrossRef]
18. Wang, Y.B.; Du, W.; Fu, A.K.; Zhang, X.P.; Huang, Y.; Lee, K.H.; Yu, K.; Li, W.F.; Li, Y.L. Intestinal microbiota and oral administration of Enterococcus faecium associated with the growth performance of new-born piglets. *Benef. Microbes* **2016**, *7*, 529–538. [CrossRef]

19. Driessen, B.; Van Beirendonck, S.; Buyse, J. The Impact of Grouping on Skin Lesions and Meat Quality of Pig Carcasses. *Animals* **2020**, *10*, 544. [CrossRef] [PubMed]
20. Henriquez-Rodriguez, E.; Tor, M.; Pena, R.N.; Estany, J. A polymorphism in the stearoyl-CoA desaturase gene promoter increases monounsaturated fatty acid content in dry-cured ham. *Meat Sci.* **2015**, *106*, 38–43. [CrossRef]
21. Ogbuagu, N.E.; Ayo, J.O.; Aluwong, T.; Akor-Dewu, M.B. L-serine improves lipid profile, performance, carcass weight and intestinal parameters in feed restricted broiler chickens during the hot-dry season. *Trop. Anim. Health Prod.* **2022**, *54*, 12. [CrossRef]
22. Lewis, C.A.; Parker, S.J.; Fiske, B.P.; McCloskey, D.; Gui, D.Y.; Green, C.R.; Vokes, N.I.; Feist, A.M.; Vander Heiden, M.G.; Metallo, C.M. Tracing Compartmentalized NADPH Metabolism in the Cytosol and Mitochondria of Mammalian Cells. *Mol. Cell* **2014**, *55*, 253–263. [CrossRef]
23. Mao, Z.; Zhang, W. Role of mTOR in Glucose and Lipid Metabolism. *Int. J. Mol. Sci.* **2018**, *19*, 2043. [CrossRef]
24. Jiang, J.; Jin, F.; Lin, G.; Xiong, Y.L. Modulation of muscle antioxidant enzymes and fresh meat quality through feeding peptide-chelated trace minerals in swine production. *Food Biosci.* **2021**, *42*, 101191. [CrossRef]
25. Zhou, X.; He, L.; Wu, C.; Zhang, Y.; Wu, X.; Yin, Y. Serine alleviates oxidative stress via supporting glutathione synthesis and methionine cycle in mice. *Mol. Nutr. Food Res.* **2017**, *61*, 1700262. [CrossRef]
26. Wang, Y.; Li, Y.; Xie, J.; Zhang, Y.; Wang, J.; Sun, X.; Zhang, H. Protective effects of probiotic Lactobacillus casei Zhang against endotoxin- and d-galactosamine-induced liver injury in rats via anti-oxidative and anti-inflammatory capacities. *Int. Immunopharmacol.* **2013**, *15*, 30–37. [CrossRef] [PubMed]
27. Chen, C.; Fang, S.; Wei, H.; He, M.; Fu, H.; Xiong, X.; Zhou, Y.; Wu, J.; Gao, J.; Yang, H.; et al. Prevotella copri increases fat accumulation in pigs fed with formula diets. *Microbiome* **2021**, *9*, 175. [CrossRef] [PubMed]
28. Petersen, C.; Bell, R.; Kiag, K.A.; Lee, S.H.; Soto, R.; Ghazaryan, A.; Buhrke, K.; Ekiz, H.A.; Ost, K.S.; Boudina, S.; et al. T cell-mediated regulation of the microbiota protects against obesity. *Science* **2019**, *365*, eaat9351. [CrossRef] [PubMed]
29. Tang, S.; Xin, Y.; Ma, Y.; Xu, X.; Zhao, S.; Cao, J. Screening of Microbes Associated With Swine Growth and Fat Deposition Traits Across the Intestinal Tract. *Front. Microbiol.* **2020**, *11*, 586776. [CrossRef] [PubMed]
30. Izzo, L.T.; Trefely, S.; Demetriadou, C.; Drummond, J.M.; Mizukami, T.; Kuprasertkul, N.; Farria, A.T.; Nguyen, P.T.T.; Murali, N.; Reich, L.; et al. Acetylcarnitine shuttling links mitochondrial metabolism to histone acetylation and lipogenesis. *Sci. Adv.* **2023**, *9*, 20. [CrossRef] [PubMed]
31. Chen, Z.; Sun, Y.; Chen, L.; Zhang, Y.; Wang, J.; Li, H.; Yan, X.; Xia, L.; Yao, G. Differences in meat quality between Angus cattle and Xinjiang brown cattle in association with gut microbiota and its lipid metabolism. *Front. Microbiol.* **2022**, *13*, 988984. [CrossRef]
32. Wang, B.; Wang, Y.J.; Zuo, S.X.; Peng, S.J.; Wang, Z.J.; Zhang, Y.J.; Luo, H.L. Untargeted and Targeted Metabolomics Profiling of Muscle Reveals Enhanced Meat Quality in Artificial Pasture Grazing Tan Lambs via Rescheduling the Rumen Bacterial Community. *J. Agric. Food Chem.* **2021**, *69*, 846–858. [CrossRef] [PubMed]
33. Goodarzi, P.; Habibi, M.; Roberts, K.; Sutton, J.; Shili, C.N.; Lin, D.; Pezeshki, A. Dietary Tryptophan Supplementation Alters Fat and Glucose Metabolism in a Low-Birthweight Piglet Model. *Nutrients* **2021**, *13*, 2561. [CrossRef] [PubMed]
34. Xiao, P.; Goodarzi, P.; Pezeshki, A.; Hagen, D.E. RNA-seq reveals insights into molecular mechanisms of metabolic restoration via tryptophan supplementation in low birth weight piglet model. *J. Anim. Sci.* **2022**, *100*, skac156. [CrossRef] [PubMed]
35. Zubiri-Gaitán, A.; Blasco, A.; Hernández, P. Plasma metabolomic profiling in two rabbit lines divergently selected for intramuscular fat content. *Commun. Biol.* **2023**, *6*, 893. [CrossRef]
36. Niu, J.K.; Liu, X.; Xu, J.Y.; Li, F.; Wang, J.C.; Zhang, X.X.; Yang, X.; Wang, L.; Ma, S.; Li, D.F.; et al. Effects of Silage Diet on Meat Quality through Shaping Gut Microbiota in Finishing Pigs. *Microbiol. Spectr.* **2023**, *11*, 19. [CrossRef]
37. Wu, C.; Lyu, W.; Hong, Q.; Zhang, X.; Yang, H.; Xiao, Y. Gut Microbiota Influence Lipid Metabolism of Skeletal Muscle in Pigs. *Front. Nutr.* **2021**, *8*, 675445. [CrossRef]
38. Bäckhed, F.; Ding, H.; Wang, T.; Hooper, L.V.; Koh, G.Y.; Nagy, A.; Semenkovich, C.F.; Gordon, J.I. The gut microbiota as an environmental factor that regulates fat storage. *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 15718–15723. [CrossRef] [PubMed]
39. Choi, Y.; Kwon, Y.; Kim, D.-K.; Jeon, J.; Jang, S.C.; Wang, T.; Ban, M.; Kim, M.-H.; Jeon, S.G.; Kim, M.-S.; et al. Gut microbe-derived extracellular vesicles induce insulin resistance, thereby impairing glucose metabolism in skeletal muscle. *Sci. Rep.* **2015**, *5*, 15878. [CrossRef]
40. Virtue, A.T.; McCright, S.J.; Wright, J.M.; Jimenez, M.T.; Mowel, W.K.; Kotzin, J.J.; Joannas, L.; Basavappa, M.G.; Spencer, S.P.; Clark, M.L.; et al. The gut microbiota regulates white adipose tissue inflammation and obesity via a family of microRNAs. *Sci. Transl. Med.* **2019**, *11*, 13. [CrossRef]