Animal Nutrition 20 (2025) 110–119

Contents lists available at ScienceDirect

Animal Nutrition

journal homepage: http://www.keaipublishing.com/en/journals/aninu/

Original Research Article

Glutamate increases the lean percentage and intramuscular fat content and alters gut microbiota in Shaziling pigs

Changbing Zheng [a, b], Mengliao Wan [a, b], Qiuping Guo [a], Yehui Duan [a, c, *], Yulong Yin [a, c, *]

[a] CAS Key Laboratory of Agro-ecological Processes in Subtropical Region, Hunan Provincial Key Laboratory of Animal Nutritional Physiology and Metabolic Process, National Engineering Laboratory for Pollution Control and Waste Utilization in Livestock and Poultry Production, Institute of Subtropical Agriculture, Chinese Academy of Sciences, Changsha 410125, China
[b] College of Animal Science and Technology, Hunan Agricultural University, Changsha 410128, China
[c] University of Chinese Academy of Sciences, Beijing 100039, China

ARTICLE INFO

Article history:
Received 1 December 2023
Received in revised form
9 July 2024
Accepted 26 July 2024
Available online 5 November 2024

Keywords:
Glutamate
Shaziling pig
Lean percentage
Intramuscular fat content
Spirochaetota
Acetic acid

ABSTRACT

This study aimed to explore the effects of glutamate (Glu) supplementation on the growth performance, carcass traits, meat quality, composition of amino acids and fatty acids in the longissimus dorsi muscle, and the colonic microbial community of Shaziling pigs. A total of 48 healthy male Shaziling pigs (150 d, 31.56 ± 0.95 kg) were randomly assigned to two groups, and fed a basal diet with no supplement (control group) or supplemented with 1% Glu (Glu group) for 51 d, and 6 pigs per group were finally slaughtered. Glu significantly increased the average daily gain ($P = 0.039$), lean percentage ($P = 0.023$), and intramuscular fat (IMF) content ($P = 0.015$), and decreased the fat percentage ($P = 0.021$) of Shaziling pigs. In the muscle, Glu increased the concentrations of inosine-5′-monophosphate ($P = 0.094$), Fe ($P = 0.002$), Cu ($P = 0.052$), and monounsaturated fatty acids (MUFAs) ($P = 0.024$), and decreased the content of C18:2n6 ($P = 0.011$), n-6 polyunsaturated fatty acids (n-6 PUFAs) ($P = 0.014$), and PUFAs ($P = 0.014$). Moreover, Glu significantly upregulated the mRNA expression of adipogenesis-related genes (*FAS*, *SREBP-1C*) ($P = 0.032$, $P = 0.026$) and muscle growth-related genes (*MyHCIIb*, *MyHCIIx*) ($P = 0.038$, $P = 0.019$) in the muscle, and increased the relative abundance of Spirochaetota ($P < 0.001$) and the acetic acid content in the colon ($P = 0.039$). Correlation analysis indicated that the acetic acid content was positively correlated with the relative Spirochaetota abundance and the IMF content, and a negative trend with the fat percentage of Shaziling pigs. In conclusion, these results indicated that Glu could simultaneously increase the lean percentage and IMF content and decrease the fat percentage of Shaziling pigs, and these beneficial effects may be related to increased colonic Spirochaetota abundance and acetic acid concentrations.

1. Introduction

Pork possesses high nutritional value and is one of the most consumed meats worldwide (Matarneh et al., 2021). However, pork quality is declining due to the excessive pursuit of growth rate and lean percentage of pigs. Therefore, the current trend is to produce safe and high-quality pork as the consumer demands. Compared to the Western commercial pig breeds, the Chinese indigenous pig breeds are favored by consumers due to their premium meat quality and attractive flavor (Li et al., 2021). Among them, the Shaziling pig is such an indigenous breed known for superior meat quality and sensory attributes as manifested by high contents of intramuscular fat (IMF), amino acids and fatty acids (Duan et al., 2023; Song et al., 2022). However, what cannot be ignored is that these pigs grow slowly and have a lower lean percentage and a high fat content (Song et al., 2022), necessitating a simultaneous improvement in carcass traits and meat quality.

* Corresponding authors.
E-mail addresses: duanyehui@isa.ac.cn (Y. Duan), yinyulong@isa.ac.cn (Y. Yin).
Peer review under the responsibility of Chinese Association of Animal Science and Veterinary Medicine

Production and Hosting by Elsevier on behalf of KeAi

https://doi.org/10.1016/j.aninu.2024.07.010

One promising strategy that may help achieve this objective is addition of certain amino acids to diets. Evidence for this is provided by observations that dietary inclusion of branched chain amino acids (Zhang et al., 2022), glycine (Zhong et al., 2021), arginine (Hu et al., 2017b; Madeira et al., 2015), or serine (Zhou et al., 2021) improves meat quality. Apart from these amino acids, glutamate (Glu) has gained our attention since Glu is the top ranked metabolite enriched in metabolic pathways for the Shaziling pig meat flavor. Moreover, the muscular Glu concentration is positively correlated with total essential amino acids, flavor amino acids, and sweet amino acids of Shaziling pigs (Duan et al., 2023). Based on these findings, it is hypothesized that Glu may play a key role in the muscle development and hence meat quality in Shaziling pigs.

Traditionally, Glu has been classified as a nutritionally "nonessential" amino acid (Wu, 2013). However, fresh insights into the effects of Glu on energy metabolism in the intestines of weaned piglets indicate that Glu is a nutritionally essential amino acid for maintaining intestinal and whole-body homeostasis in neonates (Hou et al., 2018; Qin et al., 2018; Sheng, 2015; Zhu et al., 2020). Although early research on Glu focused on the intestine (Duan et al., 2014; Rezaei et al., 2013), more recent studies have highlighted its impact on muscle and adipose tissues. Since the Glu requirement by muscle cannot be fully met through intramuscular synthesis of Glu (Ytrebø et al., 2006), it is necessary to add a large amount of Glu to the diet to support muscle growth. Indeed, recent research using a lipopolysaccharide-challenged piglet model (Duroc × Large White × Landrace) has provided direct evidence of the muscle growth-promoting effects of dietary Glu supplementation (1%) (Kang et al., 2017). Apart from its functions in muscle growth of pigs, Glu supplementation (1%) also reduces backfat thickness and increases IMF content in growing-finishing pigs (Duroc × Landrace × Yorkshire) (Hu et al., 2017a; Kong et al., 2015). These findings open up the possibility of adding appropriate Glu (1%) to diets of pigs to increase the lean percentage and improve meat quality. However, there is a paucity of data on the role of dietary Glu supplementation in Shaziling pigs.

Since 150 to 210 d of age seem to be the critical window for meat quality in Shaziling pigs (Song et al., 2022), we conducted a study using 150-d-old Shaziling pigs with a hypothesis that dietary Glu supplementation during the window period could improve growth performance, carcass traits, meat quality, amino acid and fatty acid composition of the longissimus dorsi muscle, and alter the colonic microbial community of Shaziling pigs.

2. Materials and methods

2.1. Animal ethics statement

All animal procedures of this study were performed according to the Animal Care Committee of the Institute of Subtropical Agriculture, Chinese Academy of Sciences, and adhered to the Chinese guidelines on experimental protocols and animal welfare (ISA-2020-023).

2.2. Experimental protocols

A total of 48 healthy male Shaziling pigs with a similar weight (150 d, 31.56 ± 0.95 kg) were randomly allocated to two groups. Each group had six replicates of four pigs per replicate. Pigs were fed either a basal diet with no supplement (control [Con] group), or that supplemented with 1% Glu. The composition and nutrient levels of diets are shown in Table 1. The nutritional level of the diets meets the nutritional needs of Shaziling pig (China National Standard, NY/T 2826-2015 in Chinese, Supplementary file). All Shaziling pigs are raised under the same environmental conditions with the diets and water available all the time for 51 d.

Table 1
Composition and nutrient levels of the diets for Shaziling pigs (air-dried basis, %).

Item	Diets[1]	
	Con	Glu
Ingredients		
Corn	69.45	71.40
Soybean meal	14.50	12.10
Wheat bran	13.00	12.45
Glu	0.00	1.00
$CaHPO_4$	0.73	0.73
Stone powder	1.02	1.02
Salt	0.30	0.30
Premix[2]	1.00	1.00
Total	100.00	100.00
Nutrient levels[3]		
Digestible energy, MJ/kg	13.31	13.20
Crude protein	13.27	13.14
Ether extract	1.96	1.87
Crude fibre	3.20	3.09
Calcium	0.63	0.61
Total phosphorus	0.51	0.49
Available phosphorus	0.25	0.25
Glu	2.42	3.25

Glu = glutamate.
[1] Con group: basal diet, Glu group: basal diet supplemented with 1% Glu.
[2] Premix supplied per kilogram of feed: 2200 IU, vitamin A, 220 IU, vitamin D_3, 0.5 mg vitamin K_3, 17.5 μg vitamin B_{12}, 3.5 mg riboflavin, 30 mg niacin, 10 mg d-pantothenic acid, 0.05 mg biotin, 0.3 mg folic acid, 1.0 mg thiamine, 7 mg pyridoxine, and 4.0 mg ethoxyquin, 150 mg Fe, 100 mg Zn, 30 mg Mn, 25 mg Cu, 0.5 mg I, 0.3 mg Co, and 0.3 mg Se.
[3] Digestible energy and available phosphorus were calculated, and other nutrient levels were actually measured.

2.3. Chemical analysis

Crude protein (N × 6.25) of the diet was measured according to the China National Standard (GB/T 6432-2018) using continuous flow analyzer (Auto Analyzer 3-AA3, Seal Analytic, American). Ether extract was measured according to the China National Standard (GB/T 6433-2006) by using automatic fat analyzer (SOX416, Gerhardt, Germany). Crude fibre was measured according to the China National Standard (GB/T 6434-2022) using automatic fiber analyzer (FT12, Gerhardt, Germany). The total phosphorus and calcium were measured according to the British National Standard (NF V18-213-2007) using Agilent 5100 ICP-OES (5100, Agilent, American). The Glu content was measured according to the China National Standard (GB/T 18246-2019) using an Amino Acid Analyzer L-8900 (L8900, Hitachi, Japan). Digestible energy and available phosphorus were calculated according to the China Feed Database (2021).

2.4. Growth performance

Pigs were weighed on the 1st and 51st day on an empty stomach, and recorded as the initial body weight (initial BW) and final body weight (final BW), respectively. During the trial, feed intake was recorded daily in each pen to calculate the average feed intake per pig. The initial BW, final BW and feed intake per pig were used to calculate the average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR) (Zheng et al., 2022a).

2.5. Sample collection

At the end of the trial, one pig close to the average BW of pigs in each replicate was chosen, fasted overnight (12 h), and then

slaughtered according to standard commercial procedures. Then, the blood was collected to obtain serum, and the longissimus dorsi muscles were rapidly sampled and quickly frozen by liquid nitrogen. The colonic chyme was quickly collected from the middle of colon by sterile tubes. Finally, all the samples were stored in the −80 °C refrigerator for later experiments.

2.6. Carcass traits

The left side of the carcass was weighed and then dissected into skeletal muscle and fat. The fat mass and muscle mass were weighed, and recorded to calculate the lean percentage and fat percentage; the loin-eye area was the cross-sectional area of the longissimus dorsi muscle between the 6th and 7th ribs of the carcass (Li et al., 2018).

2.7. Meat quality

The pH values were determined with a pH meter (pH-STAR, SFK-Technology, Denmark) at 45 min and 24 h post-mortem. Meat color was measured using a chromameter (CR-410, Kinica Minolta Sensing Inc., Osakam, Japan) at two different locations to obtain the L* (Lightness), a* (redness), and b* (yellowness) values. The IMF content was measured using petroleum ether and the Soxhlet Extractor method. Cooking loss, drip loss, and shear force of muscles were determined as previously described (Li et al., 2018).

2.8. Measurements of muscle composition

In the longissimus dorsi muscle, the inosine-5′-monophosphate (IMP) content was measured using the high-performance liquid chromatography as previously described (Xie et al., 2023); the moisture content was measured using a vacuum freeze dryer; the contents of trace elements were determined using an inductively coupled plasma emission spectrometer (Agilent 5110 ICP-OES, USA) as previously described (Han et al., 2022).

2.9. Serum and muscular free amino acid profiles

Serum samples (750 μL) were mixed with an equal volume of 8% sulfosalicylic acid, followed by standing for 30 min at 4 °C. The mixtures were then centrifuged at 3381 × *g* at 4 °C for 5 min. Then, the supernatant was filtered by a 0.22-μm filter. The filtrate was used to analyze serum amino acid concentrations as previously described (Duan et al., 2023). As for muscular free amino acid concentrations, 0.5 g of freeze-dried longissimus dorsi muscle samples were homogenized with 25 mL of 0.01 mol/L hydrochloric overnight at 4 °C, and centrifuged at 10,000 × *g* for 15 min to obtain the supernatant. Then, the subsequent procedures were the same as the serum samples.

2.10. Muscular fatty acid composition

Lyophilized muscle samples (150 mg) were used to analyze the fatty acid composition via gas–liquid chromatography of methyl esters using an Agilent 7890A as previously described (Zhang et al., 2022).

2.11. Quantitative real-time PCR

The quantitative real-time PCR analysis was performed in longissimus dorsi muscle samples as previously described (Zheng et al., 2021a). Briefly, the total RNA was extracted by using the Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the instructions. Then, using a cDNA Synthesis Kit (Fermentas Inc., Hanover, MD, USA), the extracted RNA was reversely transcribed into cDNA, which was subsequently used to quantify the relative mRNA expression of targeted genes using the RT-PCR system. The primers' sequences are shown in Table 2. The *GAPDH* gene was used to normalize the relative mRNA expression of target genes, which were calculated based on formula $2^{-\Delta\Delta Ct}$ method.

2.12. Gut microbiota analysis

Gut microbiota in the colon of Shaziling pigs were analyzed as previously described (Zheng et al., 2022b). Based on the output data, the gut microbial α-diversity indices (including Ace, Chao1, Shannon and Simpson) and the microbiological relative abundance were determined to evaluate the richness and constituents of gut microbiota.

2.13. Short-chain fatty acids (SCFAs)

The concentration of SCFAs in the colonic digesta, including acetate, propionate, isobutyrate, butyrate, isovalerate and valerate, were measured as previously described by using an Agilent 6890A gas chromatography (Agilent Technologies, Santa Clara, CA, United States) (Duan et al., 2019).

2.14. Statistical analysis

The experiment data were preliminarily processed with Microsoft Excel, then the unpaired *t*-tests were performed using Prism7.04 (GraphPad, LaJolla, CA, USA) and used to compare the differences between groups. The test data were expressed as mean ± SEM. Significant differences from the *t*-test were marked as * for $P < 0.05$.

3. Results

3.1. Growth performance

As shown in Table 3, compared to the Con group, dietary Glu supplementation significantly increased the final BW ($P = 0.013$) and ADG ($P = 0.039$). No significant differences in FCR were observed between the two groups ($P = 0.346$).

3.2. Carcass traits

The carcass traits are shown in Table 4. Compared to the Con group, dietary Glu supplementation increased the loin-eye area ($P = 0.002$) and lean percentage ($P = 0.023$), and decreased the fat percentage ($P = 0.021$). No significant differences in the carcass yield and average backfat thickness were observed between the two groups.

3.3. Meat quality

As presented in Table 5, compared to the Con group, dietary Glu supplementation significantly increased the IMF content ($P = 0.015$). However, no differences were observed in the meat color, pH value, drip loss, shear force, and cooking loss between the two groups.

3.4. Muscular trace element content

As shown in Table 6, compared to the Con group, dietary Glu supplementation increased iron content in the longissimus dorsi muscle ($P = 0.002$) and tended to increase the copper ($P = 0.052$) and IMP contents ($P = 0.094$).

Table 2
Primers used for quantitative real-time PCR.

Genes	Forward (5′-3′)	Reverse (5′-3′)	Size, bp
ACC	GCTGGGTTGAGCGACTAATG	GGGAAACTGGCAAAGGACTG	169
FAS	CTACCTTGTGGATCACTGCATAGA	GGCGTCTCCTCCAAGTTCTG	114
LPL	CTCGTGCTCAGATGCCCTAC	GGCAGGGTGAAAGGGATGTT	148
HSL	CTCCCAACTGGAGCAGGTTT	GGCCCCAGGTGTCACTATTC	167
FATP1	GGAGTAGAGGGCAAAGCAGG	AGGTCTGGCGTGGGTCAAAG	78
FABP4	CAGGAAAGTCAAGAGCACCA	TCGGGACAATACATCCAACA	227
ATGL	CAACGCCAAGCACATCTACG	CCAGTATCACCCAGGCAGAC	80
CPT-1	ACTGTCTGGGCAAACCAAAC	CTTCTTGATGAGGCCTTTGC	170
SREBP-1C	GCGACGGTGCCTCTGGTAGT	CGCAAGACGGCGGATTTA	218
AMPKα	GCATAGTTGGGTGAGCCACA	CCTGCTTGATGCACACATGA-3	105
Sirt1	GGTTTGAAGAATGTTGCCTG	CCGTTTACTAATCTGCTCCT	114
PGC-1α	TGTGCAACCAGGACTCTGTA	CCACTTGAGTCCACCCAGAAA	152
UCP3	TGCTGGGCACCATTCTCACC	CGATCCCTTGGGCGTGTAAAG	152
MyHC I	GGCCCCTTCCAGCTTGA	TGGCTGCGCCTTGGTTT	63
MyHC IIa	TTAAAAAGCTCCAAGAACTGTTTCA	CCATTTCCTGGTCGGAACTC	109
MyHC IIb	CACTTTAAGTAGTTGTCTGCCTTGAG	GGCAGCAGGGCACTAGATGT	83
MyHC IIx	AGCTTCAAGTTCTGCCCCACT	GGCTGCGGGTTATTGATGG	79
GAPDH	CAAAGTGGACATTGTCGCCATCA	AGCTTCCCATTCTCAGCCTTGACT	123

ACC= acetyl-CoA carboxylase; *FAS*= fatty acid synthase; *HSL*= hormone-sensitive lipase; *LPL*=lipoprotein lipase; *FATP1* = fatty acid transport protein 1; *FABP4* = fatty acid binding protein 4; *ATGL*= adipose triglyceride lipase; *CPT-1* = carnitine palmitoyl transferase-1; *SREBP-1C* = Sterol regulatory element binding proteins-1c; *AMPKα* = AMP-activated protein kinase *α*; *Sirt1* = Silent information regulator 1; *PGC-1α* = peroxisome proliferator-activated receptor-gamma co-activator-1alpha; *UCP3* = uncoupling protein 3; *MyHC* = myosin heavy chain; *GAPDH* = glyceraldehyde-3-phosphate dehydrogenase.

Table 3
The effects of dietary glutamate (Glu) supplementation on the growth performance of Shaziling pigs.

Item	Con	Glu	*P*-value
Initial BW, kg	31.63 ± 1.027	32.56 ± 1.720	0.651
Final BW, kg	69.60 ± 1.041	73.80 ± 0.928*	0.013
ADFI, kg/d	2.57 ± 0.061	2.64 ± 0.027	0.316
ADG, kg/d	0.75 ± 0.016	0.81 ± 0.021*	0.039
FCR	3.48 ± 0.182	3.28 ± 0.096	0.346

BW = body weight; ADFI = average daily feed intake; ADG = average daily gain; FCR = feed conversion ratio.
Dates were presented as mean ± SEM ($n = 6$).
*Values with asterisk mean significant difference compared with Con ($P < 0.05$).

Table 4
The effects of dietary glutamate (Glu) supplementation on the carcass traits of Shaziling pigs.

Item	Con	Glu	*P*-value
Carcass yield, %	64.41 ± 1.106	65.50 ± 0.890	0.466
Average backfat, mm	42.00 ± 1.262	40.80 ± 1.698	0.584
Loin eye area, cm^2	11.30 ± 0.480	13.92 ± 0.430*	0.002
Lean percentage, %	35.52 ± 0.768	38.24 ± 0.663*	0.023
Fat percentage, %	30.94 ± 0.675	28.37 ± 0.654*	0.021

Dates were presented as mean ± SEM ($n = 6$).
*Values with asterisk mean significant difference compared with Con ($P < 0.05$).

Table 5
The effects of dietary glutamate (Glu) supplementation on the meat quality of Shaziling pigs.

Item	Con	Glu	*P*-value
L*	49.74 ± 0.987	48.41 ± 0.301	0.227
a*	17.61 ± 0.372	18.12 ± 0.244	0.275
b*	8.52 ± 0.277	8.15 ± 0.267	0.355
$pH_{45\ min}$	6.65 ± 0.092	6.58 ± 0.125	0.673
$ph_{24\ h}$	5.43 ± 0.037	5.43 ± 0.019	0.954
Drip loss, %	3.16 ± 0.239	2.69 ± 0.304	0.246
Cooking loss, %	23.68 ± 1.406	26.38 ± 1.616	0.237
Shear force	57.19 ± 6.410	56.04 ± 4.682	0.894
IMF, %	3.03 ± 0.233	4.25 ± 0.346*	0.015

L* = lightness; a* = redness; b* = yellowness; IMF = intramuscular fat.
Dates were presented as mean ± SEM ($n = 6$).
*Values with asterisk mean significant difference compared with Con ($P < 0.05$).

Table 6
The effects of dietary glutamate (Glu) supplementation on longissimus dorsi muscle chemical composition of Shaziling pigs.

Item	Con	Glu	*P*-value
Moisture, %	74.33 ± 0.458	73.35 ± 0.788	0.312
Copper, μg/g	0.34 ± 0.014	0.38 ± 0.013	0.052
Iron, μg/g	4.01 ± 0.062	4.88 ± 0.206*	0.002
Zinc, μg/g	12.53 ± 0.567	13.57 ± 0.363	0.155
Manganese, ng/g	20.55 ± 1.918	24.60 ± 2.361	0.213
IMP, mg/g	3.07 ± 0.079	3.31 ± 0.104	0.094

IMP= inosine-5′-monophosphate.
Dates were presented as mean ± SEM ($n = 6$).
*Values with asterisk mean significant difference compared with Con ($P < 0.05$).

3.5. Serum and muscular amino acid profiles

As illustrated in Tables 7 and 8, compared to the Con group, Glu supplementation increased the concentration of Glu ($P = 0.046$), threonine ($P = 0.035$), tyrosine ($P = 0.031$) in the longissimus dorsi muscle. In addition, Glu treatment decreased the aspartate (Asp) content in the serum and longissimus dorsi muscle ($P = 0.050$, $P = 0.035$).

3.6. Muscular fatty acid composition

As shown in Table 9, dietary Glu supplementation increased the concentration of oleic acid (C18:1n9c) ($P = 0.038$), C20:1 ($P = 0.043$), and monounsaturated fatty acids (MUFAs) ($P = 0.024$), and decreased the content of linoleic acid (C18:2n6c) ($P = 0.011$), polyunsaturated fatty acids (PUFAs) ($P = 0.014$), and n-6 PUFAs ($P = 0.014$) as well as the ratio of PUFA to SFA in the longissimus dorsi muscle ($P = 0.040$).

3.7. mRNA expression of genes related to lipid metabolism and muscle growth in the longissimus dorsi muscle

As depicted in Fig. 1, dietary Glu supplementation upregulated the mRNA expression levels of fatty acid synthetase (*FAS*) ($P = 0.032$), sterol regulatory element-binding proteins-1C (*SREBP-1C*) ($P = 0.026$), myosin heavy chain IIb (*MyHC IIb*) ($P = 0.038$), and *MyHC IIx* ($P = 0.019$) in the longissimus dorsi muscle.

Table 7
The effects of dietary glutamate (Glu) supplementation on serum amino acid profiles of Shaziling pigs (μg/mL).

Item	Con	Glu	*P*-value
EAAs			
Histidine	11.76 ± 0.656	11.43 ± 0.673	0.734
Isoleucine	20.31 ± 1.278	19.13 ± 0.901	0.467
Leucine	33.82 ± 1.872	32.30 ± 1.756	0.567
Lysine	36.44 ± 3.710	28.61 ± 1.708	0.084
Methionine	5.74 ± 0.289	5.77 ± 0.347	0.956
Phenylalanine	14.66 ± 0.448	15.04 ± 0.836	0.693
Threonine	20.57 ± 1.725	20.69 ± 1.389	0.956
Valine	49.08 ± 2.380	47.33 ± 0.886	0.505
Total EAAs	192.38 ± 8.041	181.30 ± 6.813	0.278
NEAAs			
Alanine	31.13 ± 2.507	28.87 ± 1.651	0.468
Arginine	35.25 ± 2.960	35.58 ± 2.335	0.933
Cysteine	3.20 ± 0.510	3.56 ± 0.181	0.520
Aspartate	6.02 ± 0.237	5.21 ± 0.276*	0.050
Glutamate	39.14 ± 1.550	39.40 ± 2.422	0.929
Glycine	55.19 ± 3.429	52.41 ± 2.033	0.502
Proline	17.28 ± 0.849	17.61 ± 0.945	0.800
Serine	11.69 ± 0.792	11.86 ± 0.677	0.875
Tyrosine	14.09 ± 1.241	12.83 ± 0.395	0.355
Total NEAAs	212.99 ± 11.072	207.33 ± 5.486	0.657
Others			
Phosphatidylserine	1.39 ± 0.087	1.33 ± 0.094	0.623
Carnosine	4.28 ± 0.286	5.24 ± 0.279*	0.038
Taurine	40.12 ± 1.670	36.64 ± 1.860	0.193

Asp = aspartate; EAAs = essential amino acids; NEAAs = non-essential amino acids.
Dates were presented as mean ± SEM ($n = 6$).
*Values with asterisk mean significant difference compared with Con ($P < 0.05$).

Table 8
The effects of dietary Glu supplementation on amino acid profiles in the longissimus dorsi muscle of Shaziling pigs (μg/g).

Item	Con	Glu	*P*-value
EAAs			
Histidine	35.24 ± 4.251	40.60 ± 7.997	0.549
Isoleucine	131.74 ± 12.100	139.57 ± 8.171	0.603
Leucine	198.66 ± 14.864	206.03 ± 18.583	0.763
Lysine	120.65 ± 11.311	110.64 ± 10.911	0.538
Methionine	54.41 ± 7.432	58.81 ± 5.690	0.649
Phenylalanine	70.19 ± 5.972	68.77 ± 7.464	0.885
Threonine	78.30 ± 4.816	100.85 ± 7.882*	0.035
Valine	139.92 ± 13.890	148.77 ± 7.416	0.586
Total EAAs	829.11 ± 61.635	874.04 ± 66.443	0.631
NEAAs			
Alanine	585.76 ± 61.865	559.19 ± 27.771	0.724
Arginine	53.46 ± 7.903	56.68 ± 4.096	0.725
Cysteine	17.77 ± 3.313	19.21 ± 5.004	0.810
Aspartate	52.62 ± 5.218	35.23 ± 4.833*	0.035
Glutamate	97.80 ± 7.147	131.89 ± 13.182*	0.046
Glycine	301.39 ± 18.902	365.66 ± 50.237	0.259
Proline	54.04 ± 1.748	61.80 ± 12.686	0.558
Serine	63.35 ± 5.593	68.76 ± 4.166	0.456
Tyrosine	75.27 ± 3.045	89.49 ± 4.784*	0.031
Total NEAAs	1301.46 ± 98.882	1291.49 ± 83.420	0.940
Others			
Phosphatidylserine	33.27 ± 3.654	40.57 ± 8.682	0.456
Anserine	352.51 ± 39.761	738.95 ± 88.851*	0.003
Carnosine	17800.71 ± 1734.052	18434.41 ± 1246.157	0.773
Taurine	718.30 ± 87.560	711.04 ± 23.914	0.938

Asp = aspartate; Glu = glutamate; EAAs= essential amino acids; NEAAs= non-essential amino acids.
Dates were presented as mean ± SEM ($n = 6$).
*Values with asterisk mean significant difference compared with Con ($P < 0.05$).

3.8. Colonic gut microbiota composition

As shown in Fig. 2A, there was no difference in the α-diversity, including ACE, Chao1, Shannon, and Simpon indexes. As presented in Fig. 2B, compared to the Con group, Glu supplementation increased the Spirochaetota relative abundance ($P = 0.041$) and decreased the relative abundance of Firmicutes ($P = 0.047$).

Table 9
The effects of dietary Glu supplementation on the fatty acid composition in the longissimus dorsi muscle of Shaziling pigs (%).

Item	Con	Glu	*P*-value
C14:0	1.27 ± 0.053	1.35 ± 0.048	0.293
C16:0	26.78 ± 0.457	27.15 ± 0.515	0.601
C16:1	3.08 ± 0.164	3.17 ± 0.052	0.618
C18:0	15.24 ± 0.240	14.79 ± 0.312	0.276
C18:1n9t	0.11 ± 0.005	0.12 ± 0.005	0.490
C18:1n9c	37.66 ± 0.386	39.77 ± 0.794*	0.038
C18:2n6	10.96 ± 0.231	9.03 ± 0.579*	0.011
C20:0	0.25 ± 0.011	0.25 ± 0.015	0.961
C20:1	0.73 ± 0.064	0.92 ± 0.053*	0.043
C18:3n3	0.21 ± 0.013	0.19 ± 0.016	0.303
C20:2	0.31 ± 0.016	0.30 ± 0.023	0.844
C20:3n6	0.29 ± 0.027	0.26 ± 0.050	0.638
C20:4n6	2.76 ± 0.351	2.36 ± 0.476	0.522
SFAs[1]	43.54 ± 0.614	43.53 ± 0.830	0.995
MUFAs[2]	41.58 ± 0.360	43.97 ± 0.828	0.024
PUFAs[3]	14.24 ± 0.524	11.88 ± 0.587*	0.014
n-3 PUFA[4]	0.21 ± 0.013	0.19 ± 0.017	0.303
n-6 PUFAs[5]	14.07 ± 0.527	11.71 ± 0.578*	0.014
PUFA:SFA ratio	0.33 ± 0.016	0.27 ± 0.016*	0.040

SFAs = saturated fatty acid; MUFAs = monounsaturated fatty acids; PUFAs = polyunsaturated fatty acids.
Data are presented as mean ± SEM ($n = 6$).
*Values with asterisk mean significant difference compared with Con ($P < 0.05$).
[1] SFAs=C14:0 + C16:0 + C17:0 + C18:0 + C20:0.
[2] MUFAs = C16:1 + C18:1n9 + C20:1 + C22:1n9.
[3] PUFAs=C18:2n6c + C18:3n3 + C20:2+ C20:3n6 + C20:4n6.
[4] n-3 PUFA = C18:3n3.
[5] n-6 PUFAs=C18:2n6 + C20:3n6 + C20:4n6.

3.9. Short-chain fatty acids (SCFAs) content and their correlation analysis with the bacterial community composition, IMF content, and fat percentage

As presented in Fig. 3A, dietary Glu supplementation increased the concentration of acetic acid ($P = 0.039$). However, there was no difference in propionic acid, isobutyric acid, butyric acid, isovaleric acid, valeric acid between groups. In addition, correlation analysis showed that the acetic acid concentrations were positively correlated with the relative abundance of Spirochaetota ($P = 0.001$) and the IMF content ($P = 0.014$), and tended to be negatively correlated with the fat percentage ($P = 0.081$) (Fig. 3B).

4. Discussion

Dietary Glu is extensively catabolized in various stages of pig growth (Hou et al., 2016). Increasing evidence suggests that dietary Glu supplementation at the levels of 1% to 4% is safe and can promote the growth of weaner and finisher pigs (Rezaei et al., 2013). The current study showed that Glu supplementation increased the ADG and final BW of Shaziling pigs without adverse effects on their health. Glu improves the intestinal morphology and maintains intestinal barrier integrity in pigs, thus promoting the absorption of nutrients (Qin et al., 2018). This suggests that the growth-promoting effects of Glu on Shaziling pigs may be due to its beneficial roles in intestinal functions.

Carcass traits, such as carcass yield, backfat thickness, loin-eye area, lean percentage and fat percentage, reflect the body composition and the production performance of animals. The loin-eye area is often considered as an indicator of meat yield, and the backfat thickness reflects the fat mass (Buck et al., 1962; Ferrucci

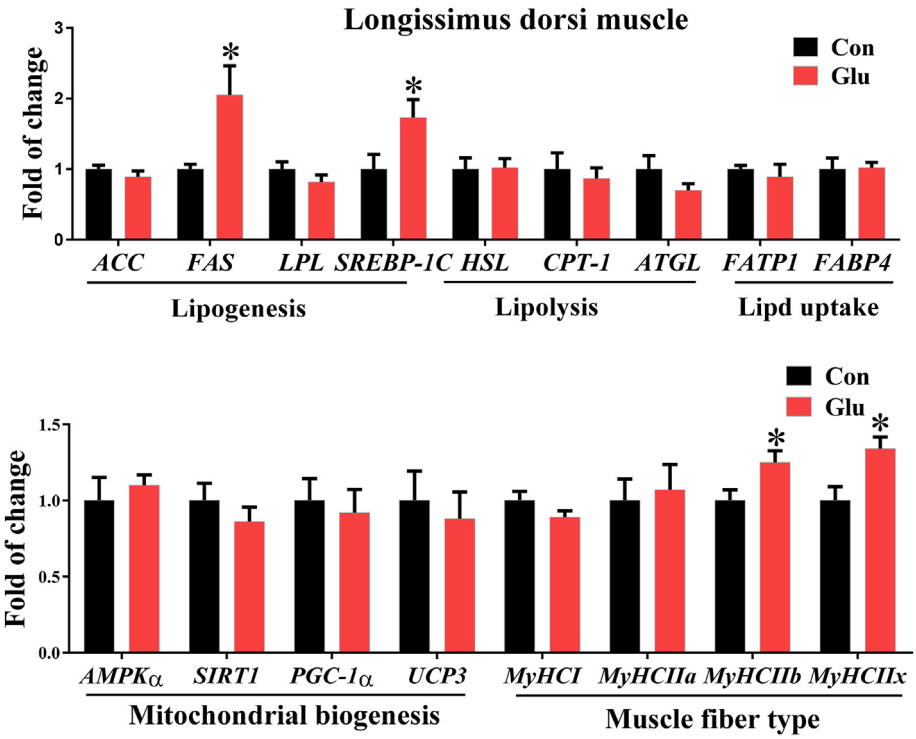

Fig. 1. The effects of dietary glutamate (Glu) supplementation on the mRNA expression levels of genes related to lipid metabolism and muscle growth in the longissimus dorsi of Shaziling pigs. Con: control group, Glu: glutamate group. Data are presented as mean ± SEM. * Values with asterisk mean significant difference compared with Con ($P < 0.05$). *ACC* = acetyl-CoA carboxylase; *FAS* = fatty acid synthase, *HSL* = hormone-sensitive lipase; *LPL* = lipoprotein lipase; *FATP1* = fatty acid transport protein 1; *FABP4* = fatty acid binding protein 4; *ATGL* = adipose triglyceride lipase; *CPT-1* = carnitine palmitoyl transferase-1; *SREBP-1C* = Sterol regulatory element binding proteins-1c; *AMPKα* = AMP-activated protein kinase α; *Sirt1* = Silent information regulator 1; *PGC-1α* = peroxisome proliferator-activated receptor-gamma co-activator-1alpha; *UCP3* = uncoupling protein 3; *MyHC* = myosin heavy chain.

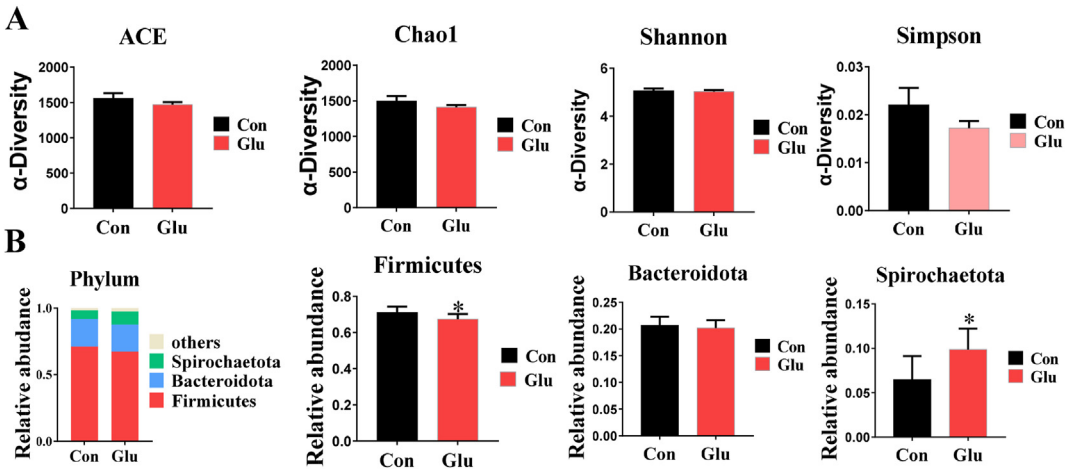

Fig. 2. The effect of dietary glutamate (Glu) supplementation on alpha-diversity and colonic gut microbiota composition. (A) ACE, Chao1, Shannon and Simpson indexes. (B) The relative abundance at the phylum level. Data are presented as mean ± SEM. Con: control group, Glu: glutamate group. * Values with asterisk mean significant difference compared with Con ($P < 0.05$).

et al., 2006). In this study, dietary Glu supplementation increased the loin-eye area and lean percentage and reduced the fat percentage of Shaziling pigs. Consistent with our results, a reduction in the average backfat thickness was also observed in Duroc × Landrace × Yorkshire pigs, although there were no significant impacts on the lean percentage (Hu et al., 2017a). These findings indicate that Glu supplementation has the potential to reduce the fat mass of both indigenous pigs and commercial lean pigs. However, the beneficial effects of Glu supplementation on the lean percentage were only observed in Shaziling pigs (indigenous pigs) but not in Duroc × Landrace × Yorkshire pigs (commercial lean pigs). Given the fact that lean percentages of indigenous pigs are lower than those of commercial lean pigs (Song et al., 2022), it is speculated that the different observations may be due to the inherent differences in the lean percentage between the two pig breeds. Another reason for the different observations may be attributable to the differences in the period of adding Glu to pigs' diets. In this study, Glu was added to the diets of Shaziling pigs

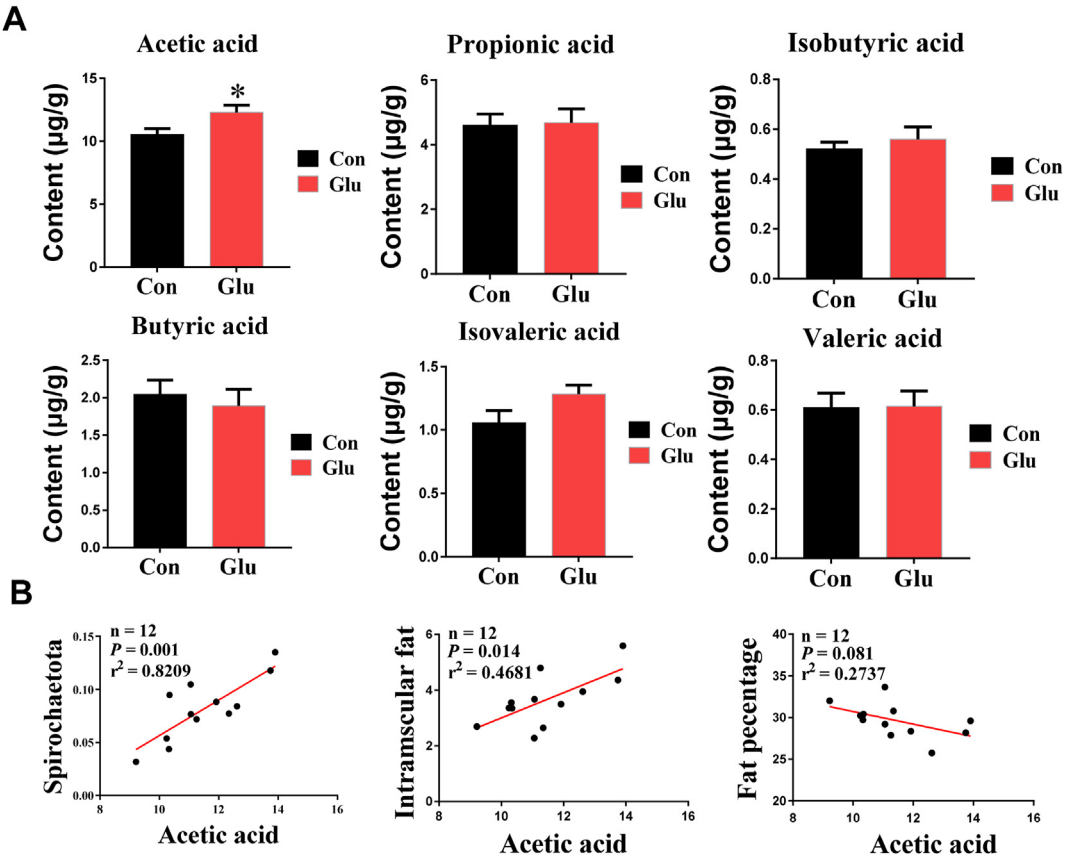

Fig. 3. The effect of dietary glutamate (Glu) supplementation on short-chain fatty acids content, and Pearson correlation analysis. (A) Concentrations of SCFAs in the colon. (B) Correlation analysis between relative Spirochaetota abundance, IMF content, fat percentage, and acetic acid. Con: control group, Glu: glutamate group. Data are presented as mean ± SEM. * Values with asterisk mean significant difference compared with Con ($P < 0.05$).

during the critical period of pork quality development (150 d, 31.56 ± 0.95 kg), whereas the initial weight of pigs used in previous studies was 77.1 ± 1.3 kg. At this stage, pigs (Duroc × Landrace × Yorkshire) mainly deposit fat rather than lean meat. Therefore, Glu supplementation mainly plays a role in regulating lipid deposition.

In the current study, we first determined the amino acid profiles in the longissimus dorsi muscle to explore the mechanisms by which Glu enhances lean tissue deposition. Evidence from a pig model showed that Glu supplementation increased the amino acid content and downregulated the mRNA levels of genes involved in protein degradation such as muscle-specific RING finger-1 and muscle atrophy F-box in the longissimus dorsi muscle (Hu et al., 2019b). Furthermore, evidence for a relationship between Glu supplementation and protein synthesis comes from the finding that dietary Glu supplementation alleviates muscle protein loss by modulating mTOR signaling pathways in LPS-challenged piglets and increases the protein content of the longissimus dorsi muscle (Kang et al., 2017). These results are consistent with the current results since dietary Glu supplementation decreased the Asp content in the serum and longissimus dorsi muscle, and increased the Glu content in the longissimus dorsi muscle. Since Glu can be synthesized from the Ala, Asp, and branched-chain amino acids in animals (Li et al., 2009), it is assumed that in response to Glu supplementation, the Asp was converted to Glu to meet the need for muscle growth, thus increasing the lean percentage of Shaziling pigs. Considering that high concentrations of serum Glu are neurotoxic (Mark et al., 2001) and the skeletal muscle has a large requirement for Glu, the different results of Glu content between serum and muscle are reasonable. Another important reason for the increased lean percentage of Shaziling pigs fed a Glu-supplemented diet may be due to the upregulated mRNA expression of *MyHC IIb* and *MyHC IIx*. Generally speaking, the rapid growth of skeletal muscle is accompanied by a higher ratio of muscle fiber types II, especially *MyHC IIb* and *MyHC IIx* (Ruusunen et al., 2004). In this study, dietary Glu supplementation led to a higher mRNA expression of *MyHC IIb* and *MyHC IIx*. These results are in agreement with the literature where 30 g/kg monosodium L-Glu (MSG, an additive form of Glu) increased the relative mRNA expression of *MyHC IIb* and *MyHC IIx* in finishing pigs (Kong et al., 2015). In summary, Glu supplementation decreased the Asp content, increased the Glu content, and upregulated the mRNA expression of *MyHC IIb* and *MyHC IIx* in the longissimus dorsi muscle, leading to enhanced lean percentage of Shaziling pigs.

Appropriate increases in IMF concentrations can enhance the acceptability, eating quality, and nutritional value of pork. When the IMF content reaches more than 3%, the meat has a good flavor, taste, and tenderness (Daszkiewicz et al., 2005; Hou et al., 2023). In this study, Glu supplementation significantly increased the IMF content of Shaziling pigs to the level of 4.25%. Similar results were obtained in finishing pigs fed high-fat diets (Duroc × Large White × Landrace), in which dietary supplementation of 30 g/kg sodium Glu increased the IMF content to the level of 4.76% (Kong et al., 2015). However, the opposite was true when finishing pigs fed a control diet (Duroc × Large White × Landrace), where Glu supplementation had no significant influence on the IMF content (Hu et al., 2017b). The percentage of fat in the diet as well as the pig breeds used may offer an explanation for the inconsistency.

Shaziling pigs, however, the increased fat deposition in response to Glu supplementation may be due to differences in Glu regulating lipid deposition in the adipose tissue and skeletal muscle.

In the present study, we also determined the fatty acid composition in the longissimus dorsi muscle of Shaziling pigs in response to Glu supplementation. Fatty acids not only affect the flavor and nutritional values of meat products, but also play a critical role in human health (Ma et al., 2015; Rhee et al., 2000). The n-3 PUFAs are important components of cell membranes and possess numerous physiological and health effects, such as esterizing cholesterol, decreasing the triglyceride (TG) content, and exerting anti-inflammatory roles; whereas the n-6 PUFAs (as the precursor of prostaglandin and leukotrienes) possess pro-inflammatory properties (Ferrucci et al., 2006). A diet with a lower n-6/n-3 PUFA ratio is thought to protect against cardiovascular diseases (Simopoulos, 2008). Moreover, PUFAs were negatively correlated with meat flavor, while MUFAs are positively correlated with meat flavor and exert a wide range of health-protective effects, such as lowering blood sugar content (Cameron et al., 2000). In this study, Glu supplementation increased the content of MUFAs and decreased the contents of n-6 PUFAs and PUFAs in the longissimus dorsi muscle. C18:1n9c and C20:1 belong to MUFAs, both of which in the longissimus dorsi muscle were significantly increased in response to Glu supplementation. We previously found that C18:1n9c was the most dominant MUFAs in the longissimus dorsi muscle of Shazilng pigs, and its content was positively correlated with IMF (Duan et al., 2023). Moreover, C18:1n9c can decrease the concentrations of low density lipoprotein, cholesterol, and lipid in blood (Su et al., 2022). C18:2n6c was the major PUFA in the longissimus dorsi muscle of Shaziling pigs and was inversely proportional to IMF (Duan et al., 2023). Therefore, the Glu supplementation-induced elevation in the IMF content may be associated with the increased C18:1n9c and decreased C18:2n6c levels in the longissimus dorsi muscle of Shaziling pigs. From the above studies, it can be inferred that Glu could improve the fatty acid composition in the longissimus dorsi muscle of Shaziling pigs, thus positively affecting the nutritional value, flavor, and eating quality of pork.

In animals, fat deposition is related to TG storage, fatty acid synthesis, lipid mobilization and the β-oxidation of fatty acids. SREBP-1C is a key nuclear transcription factor of lipid metabolism and can upregulate the mRNA expression of *acetyl-CoA carboxylase* (*ACC*) and *FAS* to promote fatty acid synthesis (Sakakibara et al., 2006). HSL is the key rate-limiting enzyme that initiates the hydrolysis of TG stored in adipose tissue (Enevoldsen et al., 2001). CPT1 is a key enzyme responsible for transporting long-and medium-chain fatty acids to enhance the β-oxidation of fatty acids (Zhao et al., 2017). AMPK, as a cellular energy sensor, is an important regulator of energy homeostasis in the body and cells (Kim et al., 2016). Numerous studies have shown that the AMPK/SIRT1 signaling pathway can activate the PGC-1α, which subsequently promotes mitochondrial biogenesis and enhances fatty acid β-oxidation (Cantó et al., 2009). In the current study, dietary Glu supplementation increased the mRNA expression of *FAS* and *SREBP-1C* in the longissimus dorsi muscle. These results indicated that Glu could increase the lipogenesis in the longissimus dorsi muscle. These findings are consistent with the result of increased IMF content in the longissimus dorsi muscle.

The literature suggests a possible role of the gut microbiota in the regulation of fat deposition in indigenous pigs (Zheng et al., 2021b). The normal gut microbiota exerts a wide range of health-promoting effects, ie., resisting enterobacter infection, producing vitamins, improving intestinal function, promoting digestion and absorption, regulating intestinal immune function, and maintaining energy metabolism homeostasis (Cho et al., 2012; Kau et al., 2011). We found that Glu supplementation increased the relative Spirochaetota abundance and the acetic acid content in the colon. Interestingly, the acetic acid content was positively associated with the relative Spirochaetota abundance and IMF content and tended to be negatively correlated with the fat percentage. However, different results were reported when looking at Duroc × Landrace × Yorkshire pigs, with Glu supplementation increasing the relative Actinobacteria abundance and the contents of butyrate and propionate (Hu et al., 2019a). An important reason for the different observations may be because of the different diets used in each experiment. The diets used in the above mentioned study had a higher crude fiber level (3.09% vs. 2.42%) and a lower protein content (13.14% vs. 14.88%) and fat (1.87% vs. 5.94%) compared with ours. In addition, acetic acid is the main SCFA, accounting for approximately 60% of the total SCFAs, and can be absorbed and utilized by many tissues (Frampton et al., 2020). Studies have shown that acetic acid is partially (approximately 40%) metabolized by the liver, and a large proportion is transported to the skeletal muscle and adipose tissue (Giron et al., 2022). Indeed, acetic acids are a possible regulator of lipid metabolism. For instance, studies in rats have shown that acetate could upregulate the mRNA expression of lipogenesis-related genes, such as *ACC* and *FAS* (Sun et al., 2023). Similarly, studies in ruminants and mice show that acetate supplementation promotes milk fat accumulation and upregulates the mRNA expression of lipogenesis-related genes of mammary epithelial cells (Qi et al., 2023; Wang, 2023). Apart from these in vivo studies, in vitro studies with 3T3-L1 preadipocytes have also shown that the peroxisome proliferator activated receptor γ (*PPARγ*)expression and lipid drops were significantly increased in response to acetate treatments (Hong et al., 2005). Studies in porcine adipocytes have shown that acetate treatment increased the expression of PPARγ and CCAAT/enhancer-binding protein alpha (C/EBPα), thus promoting adipogenic differentiation (Xu et al., 2014). Therefore, considering the described effects of Glu on the gut microbiota composition and the acetic acid content, it may be speculated that Glu supplementation reduces fat percentage and increases the IMF content of Shaziling pigs via the Spirochaetota-acetic acid axis.

5. Conclusion

Our results showed that dietary Glu supplementation increased the lean percentage and the IMF content, decreased fat percentage, and upregulated the mRNA expression of *MyHC IIb* and *MyHC IIx* in the longissimus dorsi muscle of Shaziling pigs; moreover, it increased the Spirochaetota relative abundance and acetic acid content in the colon, which was associated with increased IMF content and decreased fat percentage. These findings will facilitate better understanding of the effects of Glu on the growth and development of Shaziling pigs, and contribute to the application of Glu as a safe and efficient functional additive in Shaziling pigs.

CRediT authorship contribution statement

Changbing Zheng: Data curation, Formal analysis, Investigation, Writing – original draft. **Mengliao Wan:** Data curation, Formal analysis, Investigation, Writing – original draft. **Qiuping Guo:** Investigation, Writing – review & editing. **Yehui Duan:** Conceptualization, Project administration, Writing – review & editing. **Yulong Yin:** Funding acquisition, Resources, Validation.

Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal

interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

Acknowledgments

This study was jointly supported by the National Key Research and Development Programs of China (2023YFD1301305), the science and technology innovation Program of Hunan Province (2022RC1159), the Changsha Natural Science Funds for Distinguished Young Scholar (kq2009020) and the National Natural Science Foundation of China (U19A2037).

Appendix supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aninu.2024.07.010.

References

British National Standard. Animal feeding stuffs - determination of calcium, sodium, phosphorus, magnesium, potassium, iron, zinc, copper, manganese, cobalt, molybdenum, arsenic, lead and cadmium by ICP-AES NF V18-213-2007. Brit Stand Inst 2007.
Buck SF, Harrington G, Johnson RF. The prediction of lean percentage of pigs of bacon weight from carcass measurements. Anim Sci 1962;4(1):25–36.
Cameron N, Enser M, Nute G, Whittington F, Penman J, Fisken A, et al. Genotype with nutrition interaction on fatty acid composition of intramuscular fat and the relationship with flavour of pig meat. Meat Sci 2000;55(2):187–95.
Cantó C, Auwerx J. PGC-1alpha, SIRT1 and AMPK, an energy sensing network that controls energy expenditure. Curr Opin Lipidol 2009;20(2):98.
China Feed Database. Tables of feed composition and nutritive values in China (in Chinese), https://www.chinafeeddata.org.cn; 2021.
China National Standard. Determination of crude fat in feeds No. GB/T 6433-2006. Beijing: Standards Press of China; 2006.
China National Standard. Determination of moisture in feed stuffs No. GB/T 6435-2014. Beijing: Standards Press of China; 2018.
China National Standard. Determination of animo acid in feeds No. GB/T 6434-2022. Beijing: Standards Press of China; 2019.
China National Standard. Determination of crude fiber content in feeds. GB/T 6434-2022. Beijing: Standards Press of China; 2022.
Cho I, Blaser MJ. The human microbiome: at the interface of health and disease. Nat Rev Genet 2012;13(4):260–70.
Daszkiewicz T, Bąk T, Denaburski J. Quality of pork with a different intramuscular fat (IMF) content. Pol J Food Nutr Sci 2005;14(55):1.
Duan JL, Yin J, Wu MM, Liao P, Deng D, Liu G, et al. Dietary glutamate supplementation ameliorates mycotoxin-induced abnormalities in the intestinal structure and expression of amino acid transporters in young pigs. PLoS One 2014;9(11):e112357.
Duan Y, Zheng C, Zheng J, Ma L, Ma X, Zhong Y, et al. Profiles of muscular amino acids, fatty acids, and metabolites in Shaziling pigs of different ages and relation to meat quality. Sci China Life Sci 2023;66:1323–39.
Duan Y, Zhong Y, Xiao H, Zheng C, Song B, Wang W, et al. Gut microbiota mediates the protective effects of dietary β-hydroxy-β-methylbutyrate (HMB) against obesity induced by high-fat diets. Faseb J 2019;33(9):10019–33.
Enevoldsen L, Stallknecht B, Langfort J, Petersen L, Holm C, Ploug T, et al. The effect of exercise training on hormone-sensitive lipase in rat intra-abdominal adipose tissue and muscle. J Physiol 2001;536(3):871–7.
Ferrucci L, Cherubini A, Bandinelli S, Bartali B, Corsi A, Lauretani F, et al. Relationship of plasma polyunsaturated fatty acids to circulating inflammatory markers. J Clin Endocrinol Metab 2006;91(2):439–46.
Frampton J, Murphy KG, Frost G, Chambers ES. Short-chain fatty acids as potential regulators of skeletal muscle metabolism and function. Nat Metab 2020;2(9):840–8.
Giron M, Thomas M, Dardevet D, Chassard C, Savary-Auzeloux I. Gut microbes and muscle function: can probiotics make our muscles stronger? J Cachex Sarcopen Musc 2022;13(3):1460–76.
Han Mengmeng, Gong Saiming, Yin Yunju, Luo Jie, Jiancheng Shu, Deng Dun, et al. Effects of dietary organic selenium and iron on meat quality, antioxidant characteristics and key transporter gene expression of finishing pigs. Chin J Anim Nutr 2022;34(9):5630–41.
Hong Y-H, Nishimura Y, Hishikawa D, Tsuzuki H, Miyahara H, Gotoh C, et al. Acetate and propionate short chain fatty acids stimulate adipogenesis via GPCR43. Endocrinology 2005;146(12):5092–9.
Hou X, Zhang R, Yang M, Niu N, Wu J, Shu Z, et al. Metabolomics and lipidomics profiles related to intramuscular fat content and flavor precursors between Laiwu and Yorkshire pigs. Food Chem 2023;404(Pt A):134699.
Hou Y, Wu G. L-Glutamate nutrition and metabolism in swine. Amino Acids 2018;50(11):1497–510.
Hou Y, Yao K, Yin Y, Wu G. Endogenous synthesis of amino acids limits growth, lactation, and reproduction in animals. Adv Nutr 2016;7(2):331–42.
Hu C, Jiang Q, Zhang T, Yin Y, Li F, Deng J, et al. Dietary supplementation with arginine and glutamic acid modifies growth performance, carcass traits, and meat quality in growing-finishing pigs. J Anim Sci 2017a;95(6):2680–9.
Hu C, Li F, Duan Y, Yin Y, Kong X. Glutamic acid supplementation reduces body fat weight in finishing pigs when provided solely or in combination with arginine and it is associated with colonic propionate and butyrate concentrations. Food Funct 2019a;10(8):4693–704.
Hu CJ, Jiang QY, Zhang T, Yin YL, Li FN, Deng JP, et al. Dietary supplementation with arginine and glutamic acid modifies growth performance, carcass traits, and meat quality in growing-finishing pigs. J Anim Sci 2017b;95(6):2680–9.
Hu CJ, Li FN, Duan YH, Zhang T, Li HW, Yin YL, et al. Dietary supplementation with arginine and glutamic acid alters the expression of amino acid transporters in skeletal muscle of growing pigs. Amino Acids 2019b;51(7):1081–92.
Kang P, Wang X, Wu H, Zhu H, Hou Y, Wang L, et al. Glutamate alleviates muscle protein loss by modulating TLR4, NODs, Akt/FOXO and mTOR signaling pathways in LPS-challenged piglets. PLoS One 2017;12(8):e0182246.
Kau AL, Ahern PP, Griffin NW, Goodman AL, Gordon JI. Human nutrition, the gut microbiome and the immune system. Nature (London, U K) 2011;474(7351):327–36.
Kim J, Yang G, Kim Y, Kim J, Ha J. AMPK activators: mechanisms of action and physiological activities. Exp Mol Med 2016;48(4):e224.
Kong XF, Zhou XL, Feng ZM, Li FN, Ji YJ, Tan BE, et al. Dietary supplementation with monosodium l-glutamate modifies lipid composition and gene expression related to lipid metabolism in growing pigs fed a normal- or high-fat diet. Livest Sci 2015;180:247–52.
Li J, Zhang J, Yang Y, Zhu J, He W, Zhao Q, et al. Comparative characterization of lipids and volatile compounds of Beijing Heiliu and Laiwu Chinese black pork as markers. Food Res Int 2021;146:110433.
Li P, Knabe DA, Kim SW, Lynch CJ, Hutson SM, Wu G. Lactating porcine mammary tissue catabolizes branched-chain amino acids for glutamine and aspartate synthesis. J Nutr 2009;139(8):1502–9.
Li Y, Liu Y, Li F, Lin Q, Dai Q, Sun J, et al. Effects of dietary ramie powder at various levels on carcass traits and meat quality in finishing pigs. Meat Sci 2018;143:52–9.
Ma J, Chang W, Liu G, Zhang S, Zheng A, Li Y, et al. Effects of flavones of sea buckthorn fruits on growth performance, carcass quality, fat deposition and lipometabolism for broilers. Poultry Sci 2015;94(11):2641–9.
Madeira MS, Alfaia CM, Costa P, Lopes PA, Martins SV, Lemos JPC, et al. Effect of betaine and arginine in lysine-deficient diets on growth, carcass traits, and pork quality. J Anim Sci 2015;93:4721–33.
Mark LP, Prost RW, Ulmer JL, Smith MM, Daniels DL, Strottmann JM, et al. Pictorial review of glutamate excitotoxicity: fundamental concepts for neuroimaging. Am J Neuroradiol 2001;22(10):1813–24.
Matarneh SK, Silva SL, Gerrard DE. New insights in muscle biology that alter meat quality. Annu Rev Anim Biosci 2021;9:355–77.
Qi Y, Zheng T, Liu X, Yang S, Li Q, Shao J, et al. Sodium acetate regulates milk fat synthesis through the activation of GPR41/GPR43 signaling pathway. Front Nutr 2023;10:1098715.
Qin Q, Xu X, Wang X, Wu H, Zhu H, Hou Y, et al. Glutamate alleviates intestinal injury, maintains mTOR and suppresses TLR4 and NOD signaling pathways in weanling pigs challenged with lipopolysaccharide. Sci Rep 2018;8(1):15124.
Rezaei R, Knabe DA, Tekwe CD, Dahanayaka S, Ficken MD, Fielder SE, et al. Dietary supplementation with monosodium glutamate is safe and improves growth performance in postweaning pigs. Amino Acids 2013;44(3):911–23.
Rhee K, Waldron D, Ziprin Y, Rhee K. Fatty acid composition of goat diets vs intramuscular fat. Meat Sci 2000;54(4):313–8.
Ruusunen M, Puolanne E. Histochemical properties of fibre types in muscles of wild and domestic pigs and the effect of growth rate on muscle fibre properties. Meat Sci 2004;67(3):533–9.
Sakakibara S, Yamauchi T, Oshima Y, Tsukamoto Y, Kadowaki T. Acetic acid activates hepatic AMPK and reduces hyperglycemia in diabetic KK-A(y) mice. Biochem Biophys Res Commun 2006;344(2):597–604.
Sheng R. Regulation mechanism of acetate involved in mlik fat and milk protein synthesis in the bovine mammary epithelial cells. Inner Mongolia Agricultural University; 2015 [Ph.D].
Simopoulos AP. The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. Exp Biol Med 2008;233(6):674–88.
Song B, Zheng C, Zheng J, Zhang S, Zhong Y, Guo Q, et al. Comparisons of carcass traits, meat quality, and serum metabolome between Shaziling and Yorkshire pigs. Anim Nutr 2022;8(1):125–34.
Su Y, Sun X, Zhao S, Hu M, Li D, Qi S, et al. Dietary alfalfa powder supplementation improves growth and development, body health, and meat quality of Tibetan sheep. Food Chem 2022;396:133709.
Sun C, Li A, Wang H, Ma J, Hou J. Positive regulation of acetate in adipocyte differentiation and lipid deposition in obese mice. Nutrients 2023;15(17):3736.
Wang Y. Effect of sodium acetate supplementaion on milk fat synthesis, rumen fermentation and microflora in lactating dairy goats. Henan agricultural university; 2023 [M.D].
Wu GY. Functional amino acids in nutrition and health. Amino Acids 2013;45(3):407–11.
Xie K, Sun Y, Deng L, Yu B, Luo Y, Huang Z, et al. Effects of dietary chlorogenic acid supplementation on growth performance, meat quality, and muscle flavor substances in finishing pigs. Foods 2023;12(16).

Xu Y, Liu C, Chen S, Ye Y, Guo M, Ren Q, et al. Activation of AMPK and inactivation of Akt result in suppression of mTOR-mediated S6K1 and 4E-BP1 pathways leading to neuronal cell death in *in vitro* models of Parkinson's disease. Cell Signal 2014;26(8):1680–9.

Ytrebø LM, Sen S, Rose C, Ten Have GA, Davies NA, Hodges S, et al. Interorgan ammonia, glutamate, and glutamine trafficking in pigs with acute liver failure. Am J Physiol-Gastrointest Liver 2006;291(3):G373–81.

Zhang L, Li F, Guo Q, Duan Y, Wang W, Yang Y, et al. Balanced branched-chain amino acids modulate meat quality via adjusting muscle fiber type conversion and intramuscular fat deposition in finishing pigs. J Sci Food Agric 2022;102:3796–807.

Zhao Q, Yang R, Liu F, Wang J, Hu D-D, Yang X-W, et al. Metabolomics reveals that PPARα activation protects against lithocholic acid-induced liver injury. RSC Adv 2017;7(79):49849–57.

Zheng C, Zhang S, Duan Y, Li F, Song B, Guo Q, et al. Dietary beta-hydroxy-beta-methyl butyrate supplementation improves meat quality of Bama Xiang mini-pigs through manipulation of muscle fiber characteristics. J Funct Foods 2022a;88.

Zheng CB, Song B, Guo QP, Zheng J, Li FN, Duan YH, et al. Alterations of the muscular fatty acid composition and serum metabolome in Bama Xiang mini-pigs exposed to dietary beta-hydroxy beta-methyl butyrate. Animals 2021a;11:1190.

Zheng J, Duan Y, Zheng C, Yu J, Li F, Guo Q, et al. Long-term protein restriction modulates lipid metabolism in white adipose tissues and alters colonic microbiota of Shaziling pigs. Animals 2022b;12(21):2944.

Zheng J, Zheng C, Song B, Guo Q, Zhong Y, Zhang S, et al. HMB improves lipid metabolism of Bama Xiang mini-pigs via modulating the Bacteroidetes-acetic acid-AMPKα axis. Front Microbiol 2021b;12:736997.

Zhong YZ, Yan ZM, Song B, Zheng CB, Duan YH, Kong XF, et al. Dietary supplementation with betaine or glycine improves the carcass trait, meat quality and lipid metabolism of finishing mini-pigs. Anim Nutr 2021.

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(2):384–92.

Zhu M, Qin YC, Gao CQ, Yan HC, Wang XQ. l-Glutamate drives porcine intestinal epithelial renewal by increasing stem cell activity via upregulation of the EGFR-ERK-mTORC1 pathway. Food Funct 2020;11(3):2714–24.