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Original Research Article

Effects of main active components of rosemary on growth performance, meat quality and lipid metabolism in finishing pigs

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ABSTRACT

Rosemary extracts have been widely used as feed additives in recent years. This study aimed to investigate the effects of rosmarinic acid (RA) and ursolic acid (UA), the main active components of rosemary, on growth performance, meat quality and lipid metabolism in finishing pigs. A total of 72 finishing pigs (Landrace; initial age of 150 d) were randomly divided into 3 treatments with 8 replicates of 3 pigs each, and fed a basal diet or diet containing 500 mg/kg of RA or UA. The results showed that dietary supplementation of RA or UA had no significant effect on the growth performance and carcass traits of finishing pigs ($P > 0.05$). However, both RA and UA significantly increased the triglyceride (TG) level in soleus muscle ($P < 0.001$). Supplementation of RA increased the expression of genes related to lipogenesis and transport including fatty acid synthase (*FAS*) ($P < 0.001$), sterol regulatory element binding protein-1c (*SREBP1c*) ($P < 0.001$) and peroxisome proliferator-activated receptor γ (*PPARγ*) ($P < 0.05$), while UA increased the expression of fatty acid transport protein 1 (*FATP1*), a gene related to lipid uptake ($P < 0.05$). However, RA reduced the expression of adipogenesis-related gene acetyl-coenzyme A carboxylase α (*ACCα*) ($P < 0.01$). Characterization of cecal microbiota indicated that RA increased the microbial richness (chao 1, $P < 0.001$) and diversity (observed species, $P < 0.01$). Further analysis of the genera revealed that RA increased the relative abundance of *Bacteroides* and *g-UCG-005* ($P < 0.05$), and UA enriched *Prevotella* ($P < 0.001$). Correlation analysis showed that *g-UCG-005* was positively correlated with the expression of *FAS*, carnitine palmitoyl transferase 1B (*CPT1B*), *SREBP1c* and *PPARγ* ($P < 0.01$). In conclusion, dietary supplementation of RA or UA may increase fat deposition in muscle of finishing pigs by regulating lipid metabolism and gut microbiota.

1. Introduction

Pork is one of the most consumed meat types in many countries around the world, and the demand for high quality pork keeps increasing. The fat content and fatty acid composition of meat are important factors of meat quality, and both affect feed efficiency, meat production costs and consumer health (Liu et al., 2021b). Previous studies have shown that the expression level of lipid metabolism-related genes in adipose tissue of obese pigs is higher than that of lean pigs (Qiu et al., 2017), and local obese pigs with

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high meat quality, such as Lantang pigs of Chinese endemic breeds, have higher carcass fat content than hybrid pigs (Chen, 2010; Lu et al., 2008). In past decades, pig farmers have been more focused on satisfying the demand for industry meat products by breeding pigs with faster growth rates and higher leanness, resulting in higher leanness, lower meat quality and poorer flavor. Therefore, increasing fat deposition in pork is a long-term goal in breeding leaner pigs (Zhao et al., 2009). The production of high quality pork has become the goal of the breeding industry, and nutrition regulation is an effective and feasible means. It has been reported that natural plant resources can not only improve the carcass quality of livestock and poultry, but also increase the feed utilization (Güçlu et al., 2004). It is an efficient and safe method to precisely regulate the ability of fat deposition through natural compounds such as grape polyphenols (Aoun et al., 2011). Recent study showed that dietary supplementation of herbal bioactive components could improve broiler meat quality by altering fatty acids (Jachimowicz et al., 2022).

Rosemary (*Rosmarinus officinalis* L.) is a member of the salvia family, also known as the dew of the sea, native to the Mediterranean Sea. Previous studies have shown that rosmarinic acid (RA), ursolic acid (UA), carnosic acid and oleanolic acid are the main bioactive components in rosemary (Li et al., 2019), which exert multiple biological effects such as antioxidant and anti-inflammatory effects (Baron et al., 2021; Bianchin et al., 2020; Goncalves et al., 2022). In particular, RA has been reported to possess antioxidant, anti-inflammatory, anti-bacterial and lipid metabolism regulation properties (Ghasemzadeh Rahbardar et al., 2017; Nguyen et al., 2021; Nyandwi et al., 2021; Zhang et al., 2022a), while UA has been most extensively studied for its chemopreventative (Shanmugam et al., 2013), anti-inflammatory and anti-obesity effects (Mancha-Ramirez and Slaga, 2016). However, the effect and mechanisms of RA and UA in regulating fat deposition in finishing pigs are rarely reported. Our previous studies found that polyphenols can dose-dependently prevent high fat diet-induced obesity and hepatic fat deposition (Liu et al., 2018), and protocatechuic acid, a secondary phenolic acid, can protect against intestinal damage by regulating gut microbiota (Hu et al., 2020). In addition, dietary supplementation of plant polyphenols can improve growth and reproductive performance of pigs (Hu et al., 2022; Yuan et al., 2020). Other studies have also indicated that polyphenols can regulate the composition of gut microbes, which in turn catabolize polyphenols to release bioactive metabolites (Wang et al., 2022b), which is beneficial to the intestinal morphology of pigs (Zheng et al., 2021). In this study, the effects of RA and UA on growth performance, carcass traits, fat deposition, fatty acid profile, genes expression of lipid metabolism and gut microbial composition were investigated in Landrace finishing pigs, in order to provide theoretical basis and reference for the application of RA and UA as novel feed additives.

2. Materials and methods

2.1. Animal ethics statement

The procedures involved in animal trials were approved by the Hunan Agricultural University Institutional Animal Care and Use Committee (Permission No. 2020A34).

2.2. Animal experimental design

The trial was conducted in the Shantian base pig farm of Longhua Agricultural and Livestock Co., Ltd., following the feeding management and epidemic prevention procedures of finishing pigs. A total of 72 finishing pigs (Landrace boar; initial age of 150 d) with an average weight of 82.65 kg were randomly divided into 3 treatments with 8 replicates of 3 pigs each, and fed a basal diet or diets containing 500 mg/kg of RA or UA, respectively, according to our pilot test. The basal diet meets the NRC (2012) requirements for finishing pigs as shown in Table S1, based on a previous study (Liao et al., 2022). Briefly, values of DE, ME, standardized ileal digestibility (SID) of amino acids, and available P were calculated based on the tables of feed composition and nutritive values in China (32nd edition), while the crude protein, total Ca and total P were measured according to the National Standard of the People's Republic of China (GB/T 6432-2018, GB/T 6436-2018, and GB/T 6437-2018). The Ca concentrations of the diet were determined by potassium permanganate method. The total P contents of the diet were measured by the colorimetric method of molybdovanadate. The experimental period lasted to 184 d of age. All finishing pigs were starved for 8 h and blood samples were collected from the anterior vena cava of one pig from each replicate before the sacrifice.

2.3. Sample collection and analysis

2.3.1. Growth performance

Body weight of all the pigs was measured before (initial weight) and after (final weight) the experiment after starving for 8 h. Feed intake was recorded every day, and the average daily feed intake (ADFI), average daily gain (ADG) and feed to weight gain ratio (F:G) were calculated according to the following formula.

ADFI = total feed intake/(test days × test number);

ADG = total weight gain/(number of test days × number of tests);

F:G = average daily feed intake/average daily gain.

2.3.2. Slaughtering performance and meat quality determination

Carcass weight, slaughter rate, body straight length, carcass oblique length, back fat thickness at the junction of the 6th and 7th ribs, lion eye area of Soleus muscle (between the 5th rib and the last rib) of the left carcass, and abdominal fat rate were measured to reflect the carcass quality. Soleus muscle was collected and the hydraulic force, drip loss, cooking loss, shear force, muscle color and pH value at 45 min and 24 h after slaughter were measured according to Technical code of practice for pork quality assessment (NY/T 821-2019). A NH310 portable computer colorimeter (Osaka, Japan) was used to measure the meat color on the longest dorsal muscle at 45 min and 24 h, including brightness (L*), redness (a*), and yellow (b*). The pH value of the muscle was measured at 45 min and 24 h with a portable pH meter (pH-Star Matthäus GmbH, Pöttmes, Germany), while the pork tenderness was measured using a meat tenderness tester (Agilent 7820, USA).

2.3.3. Blood sample collection

Blood samples were collected from the front vena cava of finishing pigs with a centrifuge tube without anticoagulants, and the serum was collected after centrifugation at 1500 × *g* for 10 min after standing at room temperature for 30 min, and stored at −80 °C until analysis.

2.3.4. Fatty acid composition analysis

Freeze-dried soleus muscle powder (0.5 g) in a 50-mL centrifuge tube was extracted for 24 h with 4 mL of benzene-petroleum ether (vol:vol = 1:1) mixed solvent, and then 4 mL potassium hydroxide-methanol solution (0.4 mol/L) was added into the tube before shaking for 3 min with a vortex mixer, and stood for 30 min. Ultrapure water was then added for layering, and the upper solution was collected and dehydrated by using anhydrous sodium sulfate. Then 200 μL of the sample was diluted with 800 μL of hexane, and

filtered with a 0.22-μm filter membrane before detection. Gas chromatography was performed with a chromatographic column SP-2560 (100 m × 0.25 mm × 0.2 μm) under the following conditions: carrier gas (high purity nitrogen), flow rate (0.8 mL/min), sample volume (1 μL), split ratio (20:1), FID detector temperature (280 °C), hydrogen (30 mL/min), air (400 mL/min), column temperature (initial temperature 140 °C for 5 min, and was increased to 220 °C at 3 °C/min for 40 min). The content of each fatty acid in soleus muscle was calculated according to the peak area. Finally, the fatty acid concentration was analyzed by GC ChemStation software (Agilent Technologies, Palo Alto, CA, USA).

2.3.5. Muscle triglyceride measurement

For triglyceride measurement, the samples of soleus muscle were homogenized with normal saline solution (tissue weight:-normal saline solution = 1:9) and centrifuged at 1500 × *g* for 10 min. The concentration of triglycerides in the soleus muscle was measured with the corresponding assay kits (Nanjing Jiancheng Bioengineering Institute, China) according to the manufacturer's instructions. In addition, the concentration of protein in the homogenate was detected using a BCA Protein Assay Reagent Kit (Thermo Fisher Scientific Inc, Waltham, MA, USA).

2.3.6. Real-time fluorescence quantitative PCR

Real-time fluorescence quantitative PCR was used to analyze total RNA from soleus muscle samples according to Trizol reagent protocol (Hunan Aikerui Bioengineering Co., Ltd., Changsha, China), and the purity of total RNA was identified by spectrophotometry with optical density of 260 and 280 nm (Merinton Instrument, Inc., Ann Arbor, MI, USA). Total RNA was treated with DNase I (Hunan Aikerui Bioengineering Co., Ltd., Changsha, China) to remove DNA and reverse transcribed into complementary deoxyribonucleic acid (cDNA). PCR was carried out in a total reaction volume of 10 μL, including 5 μL of 2 × SYBR Green qPCR Master Mix (TransGen Biotech, Beijing, China), 0.2 μL of forward and reverse primers (10 μmol/L), 1 μL of cDNA template, and 3.6 μL of sterile water. PCR was performed on a LightCycler480 real-time PCR system (Rotkreuz, Switzerland). The thermal cycler parameters were as follows: 94 °C for 3 s, 45 cycles of 94 °C for 5 s and 60 °C for 30 s. Real-time fluorescence quantitative PCR was obtained from the value of the threshold period, in which the mRNA expression level (Ct) of the target gene in any unit was correlated with the internal control, and the comparative $2^{-\Delta\Delta Ct}$ method was used (Duan et al., 2019). The primer used for quantitative real-time PCR is shown in Table S2.

2.3.7. 16S rDNA gene sequencing

Cecal microbial DNA was extracted with a DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA) according to the manual. Genomic DNA purity and quality were examined on 1% agarose gel. The V3–V4 hypervariable region of the bacterial 16S rRNA gene was amplified with the primers 338F (ACTCCTACGGGAGGCAGCAG) and 806R (GGACTACHVGGGTWTCTAAT) (Munyaka et al., 2015). For each sample, a 10-digit barcode sequence was added to the 5' end of the forward and reverse primers (provided by Allwegene Company, Beijing). The PCR was carried out on a Mastercycler Gradient (Eppendorf, Germany) using 25 μL reaction volumes, containing 12.5 μL KAPA 2G Robust Hot Start Ready Mix, 1 μL forward primer (5 μM), 1 μL reverse primer (5 μM), 5 μL DNA (total template quantity is 30 ng), and 5.5 μL H_2O. Cycling parameters were 95 °C for 5 min, followed by 28 cycles of 95 °C for 45 s, 55 °C for 50 s and 72 °C for 45 s with a final extension at 72 °C for 10 min. Three PCR products per sample were pooled to mitigate reaction-level PCR biases. The PCR products were purified using a QIAquick Gel Extraction Kit (QIAGEN, Germany), quantified using Real Time PCR, and sequenced at Allwegene Company, Beijing. Deep sequencing was performed on Miseq platform at Allwegene Company (Beijing). After the run, image analysis, base calling and error estimation were performed using Illumina Analysis Pipeline Version 2.6. Qualified reads were separated using the sample-specific barcode sequences and trimmed with Illumina Analysis Pipeline Version 2.6. And then the dataset was analyzed using QIIME. The sequences were clustered into operational taxonomic units (OTU) at a similarity level of 97% (Edgar, 2013) to generate rarefaction curves and to calculate the richness and diversity indices. The Ribosomal Database Project (RDP) Classifier tool was used to classify all sequences into different taxonomic groups (Cole et al., 2009).

2.3.8. Statistical analysis

The experimental data were preliminarily sorted by Excel (2010), and then statistically analyzed by SPSS 20.0 (Chicago, IL, USA). According to the experimental design, one-way analysis of variance (ANOVA) was used to analyze the significance of differences, and then Duncan's multiple comparisons test was carried out. Mean value and standard error of the mean (SEM) were used to express the results.

Table 1
Effects of rosmarinic acid and ursolic acid on growth performance of finishing pigs.

Item	CTL	RA	UA	SEM	*P*-value
Initial weight, kg	82.24	82.15	83.29	0.837	0.831
Final weight, kg	111.74	114.61	110.63	1.292	0.439
ADG, kg/d	0.83	0.91	0.80	0.026	0.213
ADFI, kg/d	2.66	2.89	2.53	0.084	0.227
F:G	3.13	3.11	3.18	0.106	0.925

ADG = average daily gain; ADFI = average daily feed intake; F:G = feed-to-gain ratio.
CTL = basal diet; RA = basal diet containing with 500 mg/kg rosmarinic acid; UA = basal diet containing 500 mg/kg ursolic acid.
Data are expressed as means and SEM, $n = 8$.

Table 2
Effects of rosmarinic acid and ursolic acid on carcass traits of finishing pigs.

Item	CTL	RA	UA	SEM	*P*-value
Carcass weight, kg	86.01	87.22	89.50	7.200	0.109
Slaughter rate, kg	77.28	76.52	77.82	0.008	0.834
Body straight length, cm	103.09	100.25	102.98	0.572	0.084
Carcass oblique length, cm	85.26	85.56	89.34	0.810	0.066
Backfat thickness, mm	18.24	18.68	17.18	0.844	0.276
Lion eye area, cm^2	52.29	53.49	48.74	1.553	0.449
Abdominal fat rate, %	1.31	1.58	1.50	0.054	0.072
Abdominal fat, kg	1.10[b]	1.39[a]	1.29[ab]	0.046	0.032

CTL = basal diet; RA = basal diet containing with 500 mg/kg rosmarinic acid; UA = basal diet containing 500 mg/kg ursolic acid.
[a,b] Within a row, values with different superscript letters differ significantly ($P < 0.05$). Data are expressed as means and SEM, $n = 8$.

Table 3
Effects of rosmarinic acid and ursolic acid on the quality of pork.

Item	CTL	RA	UA	SEM	*P*-value
pH_{45min}	6.13	6.06	5.96	0.032	0.100
pH_{24h}	5.52	5.51	5.49	0.013	0.575
Lightness (L*)	46.35	45.50	47.89	0.470	0.109
Redness (a*)	10.40	11.11	10.21	0.320	0.492
Yellowness (b*)	7.64	8.29	8.20	0.190	0.344
Water holding capacity, %	82.13	82.76	78.67	1.463	0.489
Drip loss, %	3.37	4.02	3.48	0.493	0.856
Cooked meat rate, %	42.26	41.66	41.71	0.305	0.688
Shear force, N	102.03	105.58	94.54	4.794	0.190

CTL = basal diet; RA = basal diet containing with 500 mg/kg rosmarinic acid; UA = basal diet containing 500 mg/kg ursolic acid.
Data are expressed as means and SEM, $n = 8$.

3. Results

3.1. Effects of RA and UA on growth performance

The growth performance of finishing pigs is shown in Table 1. Dietary supplementation of RA or UA had no significant effect on the final weight, ADG, ADFI and F:G.

3.2. Effects of RA and UA on carcass quality

As shown in Table 2, dietary supplementation of RA or UA had no significant effect on carcass weight, slaughter rate, carcass straight length, carcass slant length, backfat thickness, or lion eye area, as compared with the control group ($P > 0.05$). However, RA supplementation significantly increased the weight of abdominal fat ($P = 0.032$).

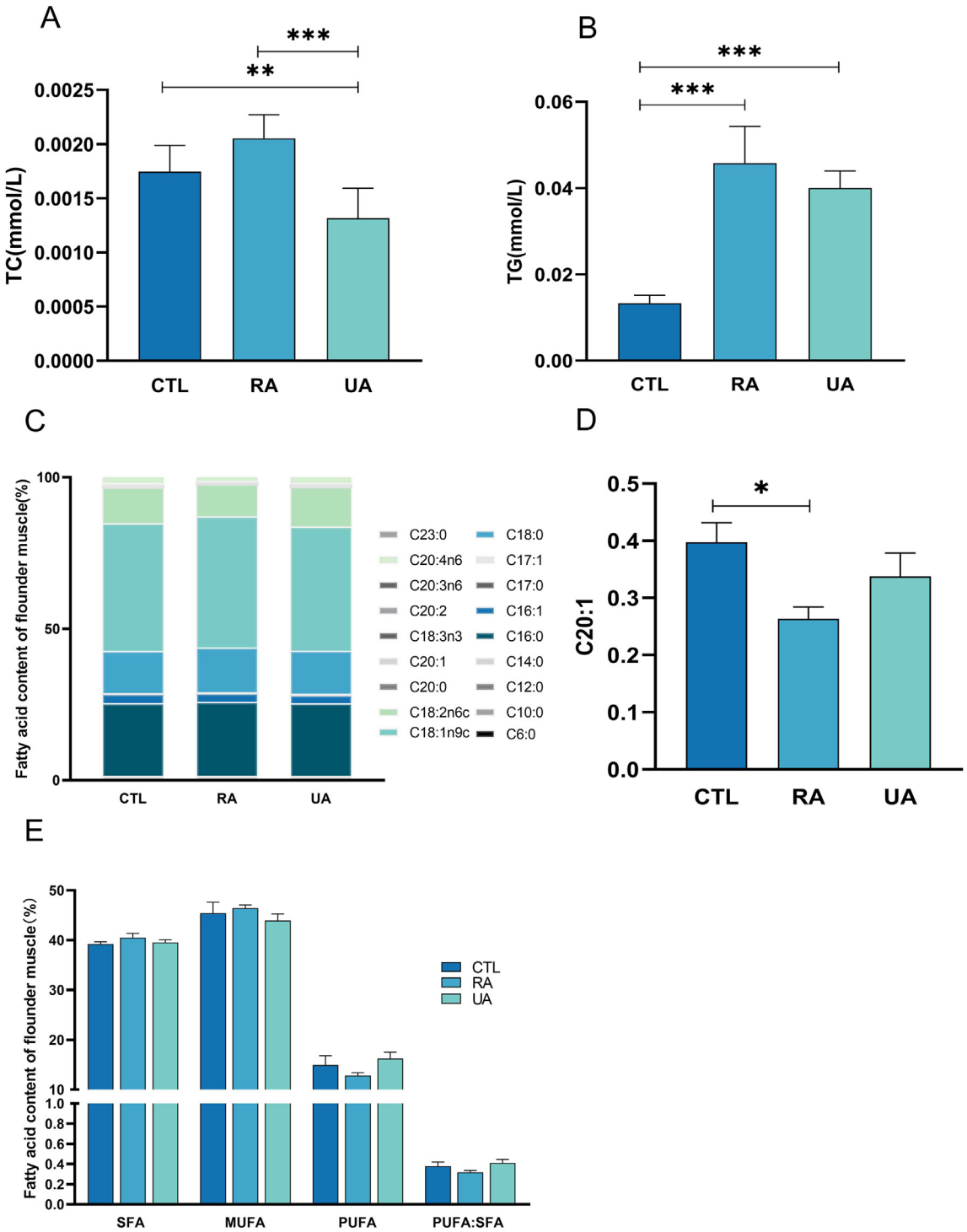

Fig. 1. Modulation of rosmarinic acid and ursolic acid on lipid levels in soleus muscle. (A) Total cholesterol (TC) level. (B) Total triglycerides (TG) level. (C) Fatty acid profile. (D) The level of C20:1. (E) Levels of saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), polyunsaturated fatty acid (PUFA) and the ratio of PUFA to SFA (PUFA:SFA). SFA = C6:0 + C14:0 + C16:0 + C18:0; MUFA = C16:1 + C18:1n9t + C20:1; PUFA = C18:2n6c + C20:2 + C20:3n6 + C20:4n6. CTL = basal diet; RA = basal diet containing 500 mg/kg rosmarinic acid; UA = basal diet containing 500 mg/kg ursolic acid. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. Data are expressed as means and SEM, $n = 6$.

3.3. Effects of RA and UA on meat quality

The effects of RA and UA on the meat quality of finishing pigs are presented in Table 3. There was no significant difference in the indices of meat quality including pH_{45min}, pH_{24h}, lightness, redness, yellowness, water holding capacity, drip loss, cooked meat rate and shear force among the three groups.

3.4. Effects of RA and UA on pig muscle lipids and fatty acid profile

As shown in Fig. 1, both dietary supplementation of RA and UA increased the level of triglycerides (TG) in the soleus muscle ($P < 0.001$, Fig. 1B), whereas UA decreased the total cholesterol (TC) level ($P < 0.01$, Fig. 1A). The fatty acid profile of soleus muscle was shown in Fig. 1C, and supplementation of RA decreased the level of C20:1 ($P < 0.05$, Fig. 1D). However, RA and UA had no significant effect on the content of saturated, monounsaturated or polyunsaturated fatty acids in soleus muscle ($P > 0.05$; Fig. 1E).

3.5. Expression levels of genes related to lipid metabolism

To further understand how RA and UA improve lipid content and fatty acid profiles in muscle, genes related to lipid metabolism were next examined in soleus muscle by RT-PCR analysis. As shown in Fig. 2, supplementation of UA increased the mRNA expression of fatty acid transport protein 1 (*FATP1*) ($P < 0.05$, Fig. 2A), while RA increased the expression of fatty acid synthase (*FAS*) ($P < 0.001$, Fig. 2A), sterol regulatory element binding protein-1c (*SREBP1c*) ($P < 0.001$, Fig. 2B) and peroxisome proliferator-activated receptor γ (*PPARγ*) ($P < 0.05$, Fig. 2B). In contrast, supplementation of RA reduced the expression of acetyl-coenzyme A carboxylase α (*ACCα*) in soleus muscle ($P < 0.01$, Fig. 2A).

3.6. Modulation of gut microbiota by RA and UA

Gut microbiota has been proved to have an important role in regulating lipid metabolism (Ko et al., 2020; Kolodziejczyk et al., 2019; Nieuwdorp et al., 2014; Wang et al., 2016), and thus the cecal microbiota of pigs was further analyzed by 16S rDNA gene sequencing. As shown in Fig. 3, supplementation of RA increased both Chao 1 index ($P < 0.001$, Fig. 3A) and observed species ($P < 0.01$, Fig. 3B), whereas UA showed no significant effect. Analysis of specific microbial phyla revealed that Firmicutes and Proteobacteria were the dominant bacteria, followed by Bacteroidetes (Fig. 3C). Both RA and UA showed no significant effect on the ratio of Firmicutes to Bacteroidetes (Fig. 3D), but the relative abundance of Proteobacteria was reduced by RA ($P < 0.01$, Fig. 3E) and UA ($P < 0.05$, Fig. 3E).

Next, the relative abundance of specific microbes was further assessed at the genus level (Fig. 4A). Dietary supplementation of RA enhanced the relative abundance of *g-Bacteroides* and *g-UCG-005* ($P < 0.05$, Fig. 4B and C), while UA increased the relative abundance of *g-Prevotella* ($P < 0.001$, Fig. 4D), as compared to the control group.

3.7. Correlation analysis of gut microbiota and lipid metabolism gene expression

The correlation analysis between the top 40 microbial genera and lipid metabolism genes are shown in Fig. 5. Microbial genera including *g-Streptococcus*, *g-UCG-005*, *g-Lachnospiraceae-NK4A136-group*, *g-Treponema*, *g-Christensenellaceae-R-7-group*, *g-Subdoligranulum*, *g-NK4A214-group*, *g-Blautia*, *g-Ruminococcus*, *g-Methanobrevibacter*, and *g-Lachnoclostridium* showed a significant correlation with the mRNA expression of lipid metabolism genes ($P < 0.05$, Fig. 5A). Based on the Spearman's correlation results (Fig. 5B), *g-UCG-005* had a positive linear correlation with the mRNA expression of *FAS*, carnitine palmitoyl transferase 1B (*CPT1B*), *SREBP1c* and *PPARγ* ($P < 0.05$, Fig. 5C).

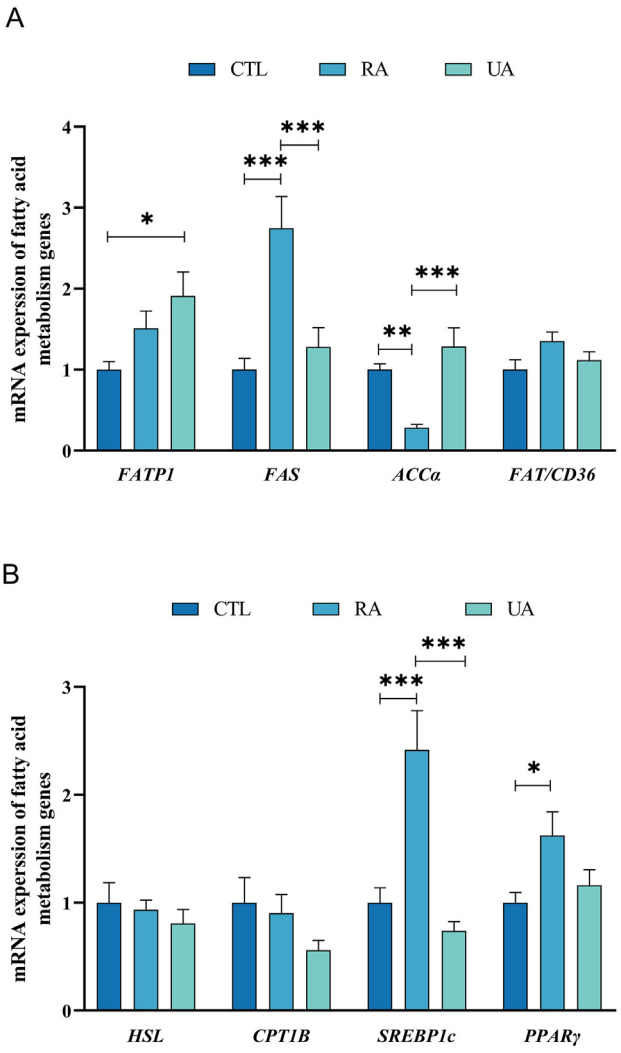

Fig. 2. Relative mRNA expression levels of key genes associated with lipid metabolism. (A) Expression of fatty acid transport protein 1 (*FATP1*), fatty acid synthase (*FAS*), acetyl-coenzyme A carboxylase α (*ACCα*) and fatty acid translocase (*FAT/CD36*). (B) Expression of hormone-sensitive lipase (*HSL*), carnitine palmitoyl transferase 1B (*CPT1B*), sterol regulatory element binding protein-1c (*SREBP1c*) and peroxisome proliferator-activated receptor γ (*PPARγ*). CTL = basal diet; RA = basal diet containing with 500 mg/kg rosmarinic acid; UA = basal diet containing 500 mg/kg ursolic acid. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. Data are expressed as means and SEM, $n = 8$.

4. Discussion

Plant extracts, especially polyphenols, have received increasing attention due to their effects against oxidative stress and lipid metabolic disorder in recent years. Rosemary is rich in polyphenolic substances including phenolic acids, flavonoids and phenoterpenes, and has been reported to have a significant effect on cognitive disorders, anti-oxidation, and metabolic improvement (Bao et al., 2020). With the improvement in living standards, consumer preferences have shifted from leaner pork to higher quality pork (Wang et al., 2021), and feed is an environmental factor that directly affects meat quality (Tai, 2022). Multiple studies have shown that dietary supplementation of plant polyphenols can improve the pH and color of meat, but reduce the indices of muscle

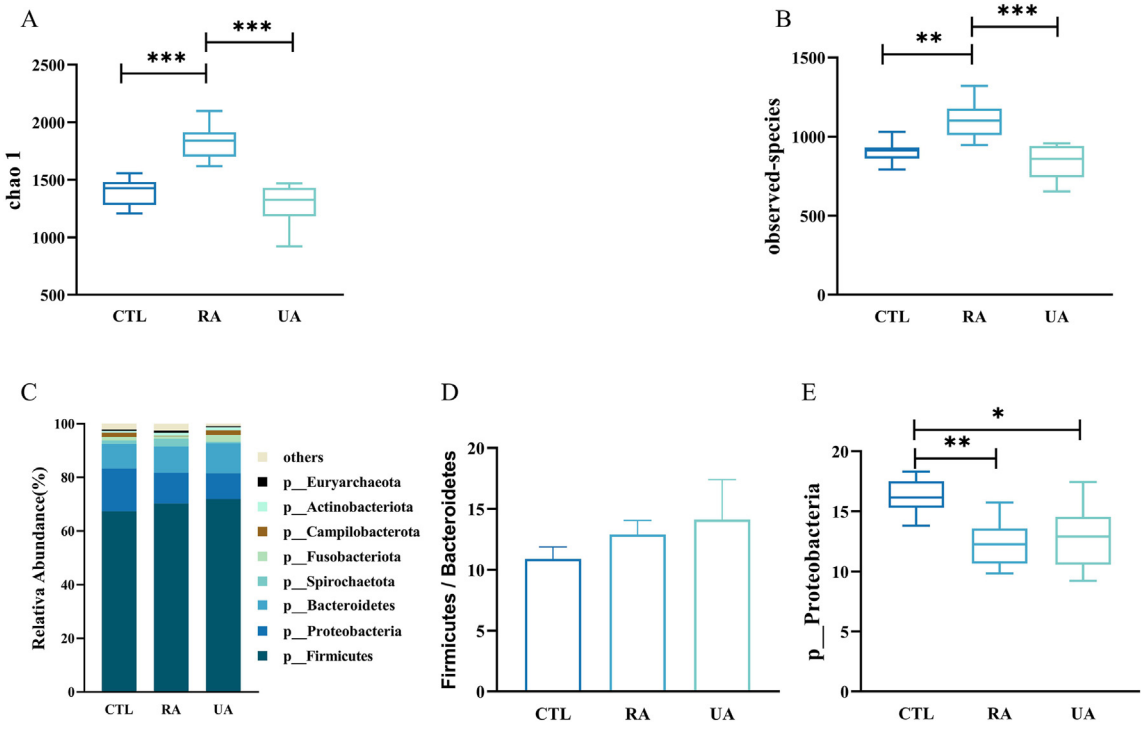

Fig. 3. Effects of rosmarinic acid and ursolic acid on cecal microbial alpha diversity and phyla. (A) Chao 1 index. (B) Observed species. (C) Microbial phyla structure. (D) The ratio of Firmicutes to Bacteroidetes. (E) The relative abundance of Proteobacteria phylum. CTL = basal diet; RA = basal diet containing with 500 mg/kg rosmarinic acid; UA = basal diet containing 500 mg/kg ursolic acid. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. Data are expressed as means and SEM, $n = 8$.

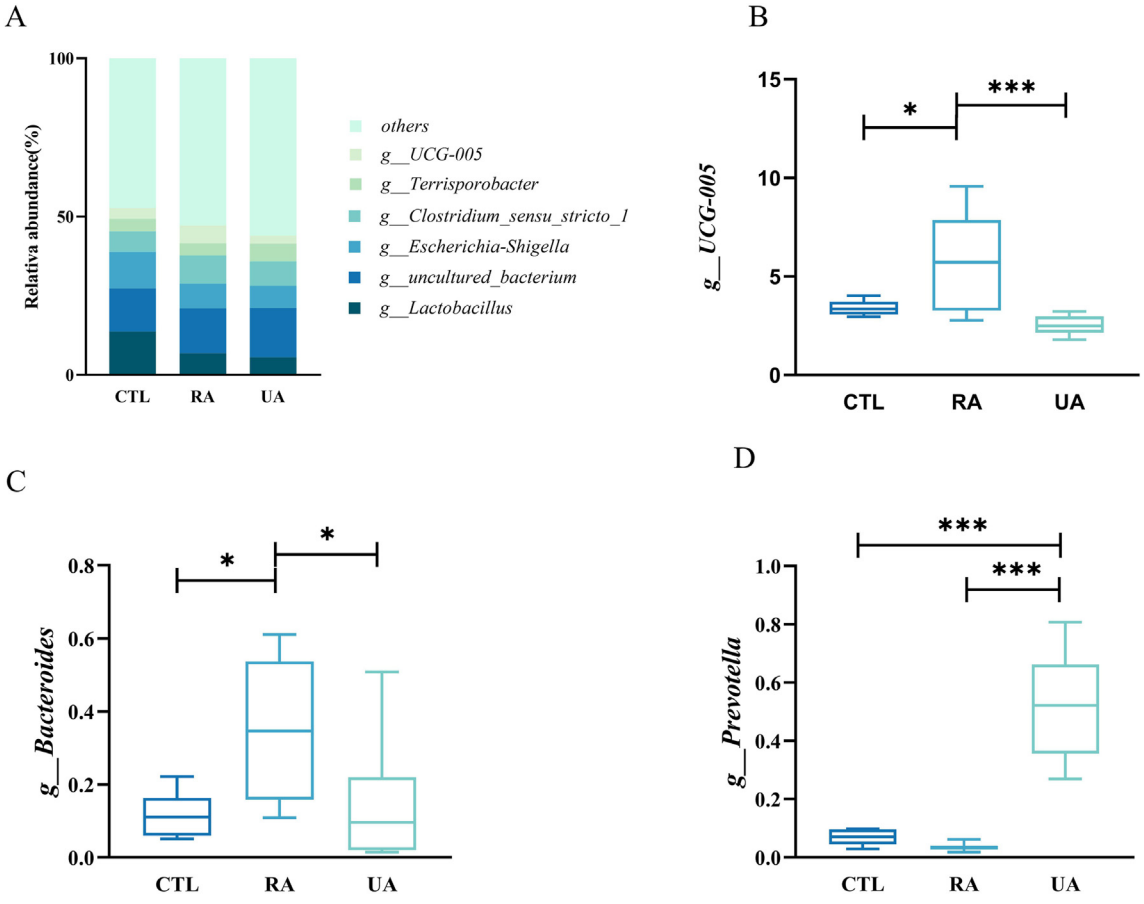

Fig. 4. Modulation of gut microbial genera by rosmarinic acid and ursolic acid. (A) Microbial genera composition. (B) The relative abundance of *g-UCG-005*. (C) The relative abundance of *g-Bacteroides*. (D) The relative abundance of *g-Prevotella*. CTL = basal diet; RA = basal diet containing with 500 mg/kg rosmarinic acid; UA = basal diet containing 500 mg/kg ursolic acid. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. Data are expressed as means and SEM, $n = 8$.

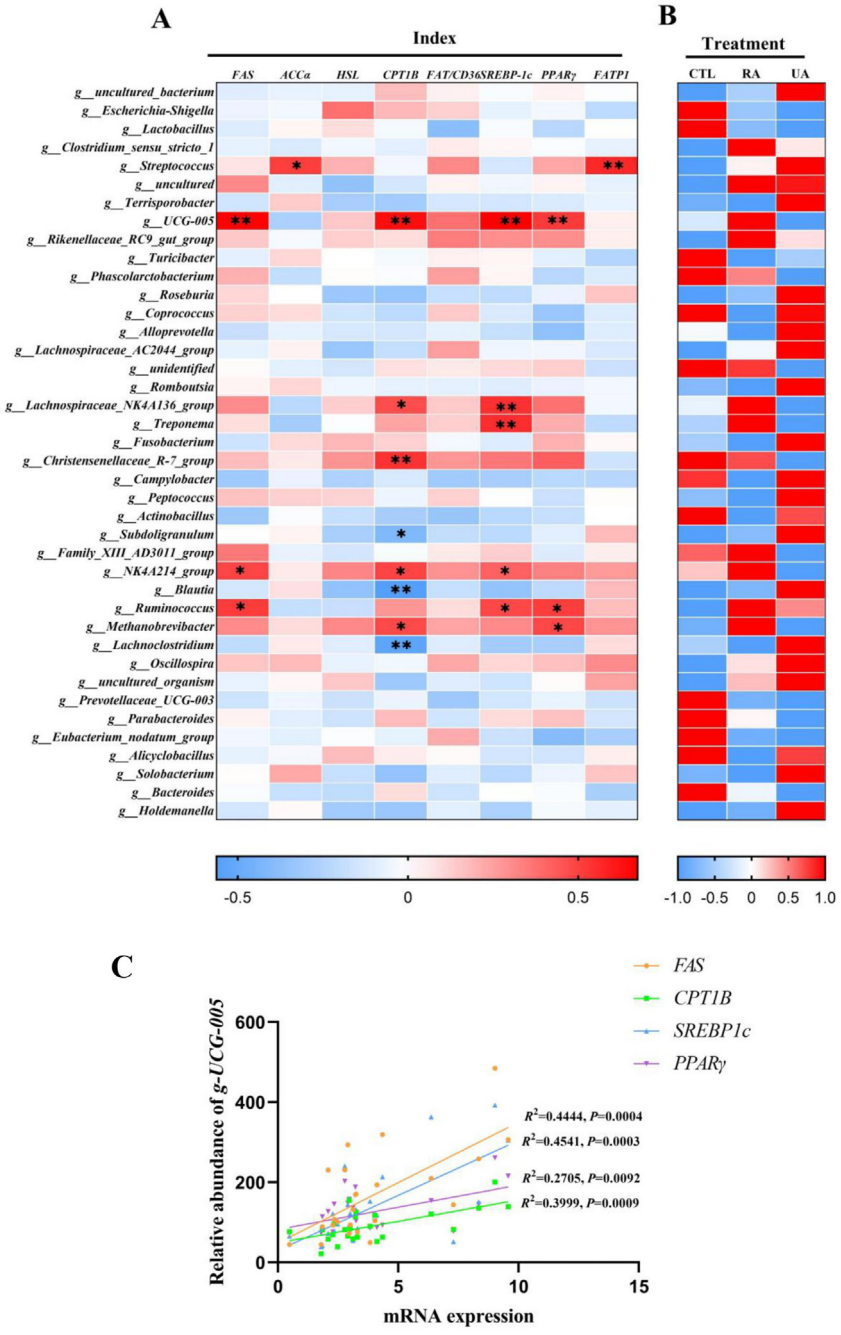

Fig. 5. Correlation analysis between gut microbiota and lipid metabolism gene expression. (A) Heatmap of Spearman's correlation between lipid metabolism gene expression and gut microbiota. (B) Heatmap of the significant different genera modulated by RA or UA. (C) Linear regression analysis between lipid metabolism gene expression and *g-UCG-005*. *FAS* = fatty acid synthase; *ACCα* = acetyl-coenzyme A carboxylase α; *HSL* = hormone-sensitive lipase; *CPT1B* = carnitine palmitoyl transferase 1B; *FAT/CD36* = fatty acid translocase; *SREBP1c* = sterol regulatory element binding protein-1c; *PPARγ* = peroxisome proliferator-activated receptor γ; *FATP1* = fatty acid transport protein 1. CTL = basal diet; RA = basal diet containing with 500 mg/kg rosmarinic acid; UA = basal diet containing 500 mg/kg ursolic acid. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. Data are expressed as means and SEM, $n = 8$.

water loss, shear force, and drip loss (Zhang et al., 2015, 2017; Zhao et al., 2018). In this study, supplementation of RA or UA showed no significant effects on growth performance and carcass quality, but increased the level of abdominal fat and TG in muscle, although UA and RA were reported to reduce TC and TG levels in mice fed a high-fat diet (Cheng et al., 2020; Nyandwi et al., 2021). Additionally, UA reduced the TC level, suggesting that RA and UA may have an effect on lipid metabolism in pigs. Previous studies have shown positive effects of rosemary extract (Yesilbag et al., 2011), RA and UA on growth performance in broiler chickens (Cheng, 2018; Shang et al., 2022), while other studies showed that dietary RA showed no significant effect on growth performance in chickens (Sierżant et al., 2021). In the current study, there was no statistically significant difference in growth performance among the groups, although the ADG was 0.83 kg/d and 0.91 kg/d in the CTL and RA goup, respectively. The experimental period (150 to 184 d of age) might be one of the potential reasons, but more data relating to dose and time-course are needed.

Fatty acids have been considered a main factor in lipid metabolism and meat quality. In this study, analysis of fatty acids

revealed that RA significantly decreased the content of C20:1 in muscle, and further analysis of genes involved in lipid metabolism revealed that RA increased the expression of *FAS*, *SREBP1c* and *PPARγ*. The expressions of *SREBP1c* and *PPARγ* are closely related to de novo lipogenesis of fatty acids (Zhong et al., 2021), while FAS is an enzyme involved in the process of fat synthesis. Therefore, RA increased fat mass in finishing pigs at least partly by up-regulating the expression of *FAS*, *SREBP1c* and *PPARγ*. *FATP1* was reported to be involved in regulating skeletal muscle fat deposition, which may be affected by fatty acid substrates, in stressed broilers (Wang et al., 2022a). In this study, dietary supplementation of UA increased the expression of *FATP1* and *ACCα*, which might have a connection with the increased TG level and decreased TC level, although the mechanisms are not clear.

Recent studies have suggested that gut microbes have a critical effect in regulating host lipid metabolism (Liu et al., 2021a) and affecting intestinal health (Blachier et al., 2017), and the fecal microbes of uncoupling protein 1 knock-in pigs participate in regulating host lipid metabolism (Pan et al., 2023). Plants rich in natural active ingredients can improve the structure of intestinal microbiota of livestock (Lin et al., 2019). Improvement in fatness traits can be achieved by many factors, including genetics, feed, and management (Zhang et al., 2022b). In addition to these well-known factors, the gut microbiota have been reported to contribute to host fat deposition in pigs (Chen et al., 2021; Lu et al., 2018; Yang et al., 2018). The genera *Alloprevotella* and *Ruminococcaceae UCG-005* were highly positively correlated with body weight and average daily gain, while the genera *Prevotellaceae UCG-001* and *Alistipes* in the cecum and *Clostridium sensu stricto 1* in the jejunum were highly positively correlated with backfat thickness and intramuscular fat (Tang et al., 2020). Growing evidence makes it clear that specific probiotic supplementation is efficient in ameliorating animal health and improving meat quality. For example, dietary inclusion of *Clostridium butyricum* and *Rhodobacter capsulatus* have been reported to improve meat quality and fatty acid profile in the broiler (Salma et al., 2007; Yang et al., 2010). It has also been reported that direct-fed probiotics can improve growth performance and feed efficiency in pigs (Kyriakis et al., 1999). In this study, supplementation of RA increased both richness (chao 1 index) and diversity (observed species) of cecal microbiota.

Further analysis at the genus level showed that UA mainly up-regulated the relative abundance of *Prevotella*. Chen et al. (2021) reported that *Prevotella* increases fat accumulation in Duroc pigs. Therefore, UA may enrich *Prevotella* and thus increase fat deposition in finishing pigs. RA mainly up-regulated the relative abundance of *g-UCG-005*, which belongs to the phylum Firmicutes, and it was found that fermented plants or citrus can regulate lipid metabolism by reducing Firmicutes to Bacteroides ratio (Wu et al., 2023). In this study, the mRNA expression of *FAS*, *CPT1B*, *SREBP1c* and *PPARγ* in muscle showed a significant correlation with the relative abundance of *g-UCG-005* in the phylum Firmicutes. Therefore, RA might improve lipid metabolism and meat quality at least partly by modulating expression of lipogenesis genes (*FAS*, *SREBP1c* and *PPARγ*).

5. Conclusion

In conclusion, dietary supplementation of RA or UA showed no significant effect on the growth performance or carcass quality of Landrace finishing pigs, but increased the content of TG in muscle. Meanwhile, UA decreased the level of TC. Analysis of genes related to lipid metabolism indicated that RA increased the expression of lipogenesis genes including *FAS*, *SREBP1c* and *PPARγ*. Characterization of cecal microbiota revealed that RA increased the microbial richness and diversity. The correlation analysis indicated that RA could increase the expression of *FAS*, *CPT1B*, *SREBP1c* and *PPARγ* in muscle potentially by up-regulating the relative abundance of *g-UCG-005* in Firmicutes phylum. These results suggest that rosemary extract can be used as a novel feed additive to improve meat quality in finishing pigs.

Author contributions

Qianjin Zhang and **Shusong Wu**: were the primary investigators in this study. **Jiatai Gong, Hongkun Xiang, Ruizhi Hu, Xizi Yang, Wentao Zhang** and **Jing Lv**: participated in the animal experiments. **Ming Liu, Xiong Deng, Xupeng Yuan, Ziyu He** and **Yixuan Jiang**: participated in sample analysis and statistical data analysis. **Jianhua He** and **Bie Tan**: revised the manuscript. **Shusong Wu**: designed this study and wrote the manuscript as corresponding author.

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.

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Appendix Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aninu.2023.05.015.

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