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Profiles of muscular amino acids, fatty acids, and metabolites in Shaziling pigs of different ages and relation to meat quality

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Pork meat is closely related to physicochemical alterations during growth and development, resulting in differences in nutritional value and meat flavor. This study aimed to evaluate the composition of amino acids, fatty acids, and metabolic profiles in the *longissimus thoracis* muscle (LM) of Shaziling pigs aged 30, 90, 150, 210, and 300 days. The results showed that the predominant fatty acids identified in the LM of Shaziling pigs were C16:0, C16:1, C18:0, C18:1n9c, and C18:2n6c. An opposite correlation was observed for C18:2n6c and n6/n3 polyunsaturated fatty acids (P<0.05). Alanine, aspartate, glutamate, D-glutamine, and D-glutamate metabolism were the main metabolic pathways for the Shaziling pig meat flavor (P<0.05). Moreover, the correlation coefficients revealed that the contents of anserine, C16:0, C16:1, and C18:1n9c were positively correlated with intramuscular fat and/or pH_{24h} and were negatively correlated with the values of L* (lightness) and b* (yellowness) (P<0.05). In conclusion, age greatly affected the meat quality of Shaziling pigs, and the contents of muscular anserine, C16:0, C16:1, and C18:1n9c might be promising indicators for better meat quality.

Shaziling pigs, meat quality, amino acids, fatty acids, metabolites

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INTRODUCTION

Pork is the most widely consumed meat worldwide, especially in China, due to its delicacy and low price. Compared with hybrid pigs, indigenous pigs have recently garnered remarkable interest from scientists and consumers owing to their better meat quality (Guo et al., 2019; Huang et al., 2020; Liu et al., 2017; Tu et al., 2021; Zhan et al., 2021; Zhu et al., 2022). The Shaziling pig, a well-known indigenous breed in China, has superior meat quality characteristics compared to those of most local pigs (Yang et al., 2016). However, limited information is available on the meat quality traits of Shaziling pigs. We previously reported that the best meat quality of Shaziling pigs is obtained from 150 to 210 days of age, and the serum L-carnitine content might be a meat quality indicator (Song et al., 2022). Despite these interesting findings, it remains unclear how the excellent meat quality traits of Shaziling pigs develop. Therefore, further research is required to advance the understanding of the meat quality traits of these pigs.

Amino acids and fatty acids in muscles are closely related

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to all meat quality aspects. Specifically, the composition of amino acids in muscle mainly determines the nutritional value and flavor of meat, whereas the composition of fatty acids in muscle largely affects edible traits, such as tenderness, juiciness, and flavor (Cameron et al., 2000; Ma et al., 2020; Wood et al., 2008). For instance, anserine (Ans) and carnosine (Car) are important meat flavor precursors (Khan et al., 2015; Wasserman and Gray, 1965). Moreover, other amino acids, such as threonine (Thr), lysine (Lys), proline (Pro), serine (Ser), alanine (Ala), and glycine (Gly), lead to the sweet taste of meat (Ma et al., 2020). Unsaturated fatty acids (UFAs) can influence the formation of volatile compounds via lipid oxidation (Cameron et al., 2000). Therefore, a better understanding of how amino acids and fatty acids affect final meat quality is required for quality optimization. The amino acid and fatty acid content in meat are influenced by many factors, such as breed, age, sex, muscle type, and the pre-slaughter treatment method (Joo et al., 2013; Jung et al., 2013). Among these factors, age is a major factor influencing the meat chemical composition and meat quality (Li et al., 2022; Liu et al., 2013; Moya et al., 2001; Wang et al., 2020). More importantly, age has been demonstrated to be superior to breed in effectively altering meat quality traits (Wang et al., 2020). However, there is little information on the correlation between meat quality traits and the amino acid and fatty acid content in Shaziling pigs of different ages.

Metabolomics has been proven to be a powerful tool for investigating the chemical composition of animal meat and evaluating its quality (Zhang et al., 2021a). For instance, nuclear magnetic resonance spectroscopy-based metabolomics has been used to identify the metabolite composition of duck muscle in relation to age (Liu et al., 2013). Wang et al. (2020) applied untargeted metabolomics approaches to investigate the effects of breed and age on duck breast meat quality and metabolic profiles. Non-targeted liquid chromatography-mass spectrometry has been used to characterize lipid-related metabolites in pig muscles (Dannenberger et al., 2017). These studies contribute to a better understanding of meat quality traits. However, there is a paucity of published data on the effects of age on the correlation between muscle metabolites and meat quality traits of Shaziling pigs. Therefore, this study aimed to (i) investigate the effects of age on the profiles of amino acids, fatty acids, and metabolites in the *longissimus thoracis* muscle (LM) of Shaziling pigs and (ii) explore the potential relationship between changes in metabolic profiles and meat quality traits of Shaziling pigs of different ages.

RESULTS

Descriptive statistics of meat quality at different ages

As shown in Table S1 in Supporting Information, the pH_{45min} values of Shaziling pigs at 90 and 150 days of age were significantly lower than those at 30, 210, and 300 days of age ($P<0.05$). The pH_{24h} value at 300 days of age was significantly higher than that at other ages ($P<0.05$). With increasing age, the values of L* (lightness) and b* (yellowness), pressure loss, and drip loss significantly decreased ($P<0.05$), while the a* (redness) value and shear force first increased and then decreased ($P<0.05$), and the intramuscular fat (IMF) first decreased and then increased ($P<0.05$). As shown in Figure S1 in Supporting Information, a notable quadratic relationship was observed between the age and pH_{24h} value ($P<0.05$). The minimum pH_{24h} value (5.54) was achieved at 103 days of age. There was no notable quadratic relationship between age and the other meat quality parameters ($P>0.05$).

Descriptive statistics of free amino acid profiles at different ages

The differences in free amino acid profiles in the LM of Shaziling pigs at different ages are shown in Table 1. With increasing age, the concentrations of leucine (Leu), isoleucine (Ile), valine (Val), His, Lys, methionine (Met), phenylalanine (Phe), Thr, total essential amino acids (EAA), Ala, arginine (Arg), glutamate (Glu), Gly, Ser, Tyr, Pro, total non-essential amino acids (NEAA), flavor amino acids (FAA), sweet amino acids (SAA), and p-serine (p-Ser) first decreased and then increased ($P<0.05$). The aspartic acid (Asp), cysteine (Cys), taurine (Tau), citrulline (Cit), and β-alanine (β-Ala) concentrations significantly decreased ($P<0.05$), ornithine (Orn) concentrations first increased and then decreased ($P<0.05$), and the Ans and Car concentrations significantly increased ($P<0.05$). There was no notable quadratic relationship between age and EAA, NEAA, FAA, and SAA ($P>0.05$). Minimal EAA (1,076 μg g^{-1}), NEAA (1,250 μg g^{-1}), FAA (673 μg g^{-1}), and SAA (1,355 μg g^{-1}) concentrations were obtained at 178, 200, 198, and 198 days of age, respectively (Figure 1). Additionally, Glu concentration was positively correlated with total EAA, NEAA, FAA, and SAA concentrations ($P<0.05$, Figure 2).

Descriptive statistics of fatty acid composition at different ages

Table 2 shows the fatty acid composition of the LM of Shaziling pigs. A total of 22 fatty acids, including 10 saturated fatty acids (SFAs), 5 monounsaturated fatty acids (MUFAs), and 7 polyunsaturated fatty acids (PUFAs), were analyzed. The SFA content ranged from 38.67% to 41.98% in the LM of Shaziling pigs at different ages, and C16:0 and C18:0 were the main SFAs. With increasing age, the percentage of C16:0 first decreased and then increased, whereas the percentage of C18:0 first increased and then decreased

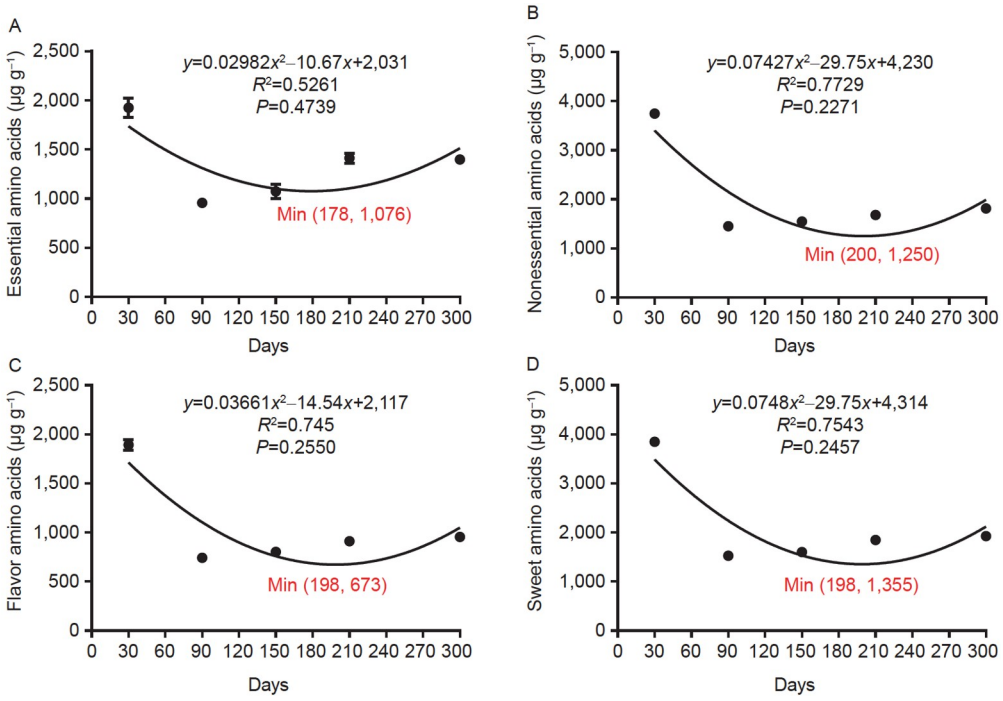

Figure 1 Quadratic regression analysis for the relations between days of age and essential amino acids (A), non-essential amino acids (B), flavor amino acids (C), and sweet amino acids (D) in the *longissimus thoracis* muscle of Shaziling pigs.

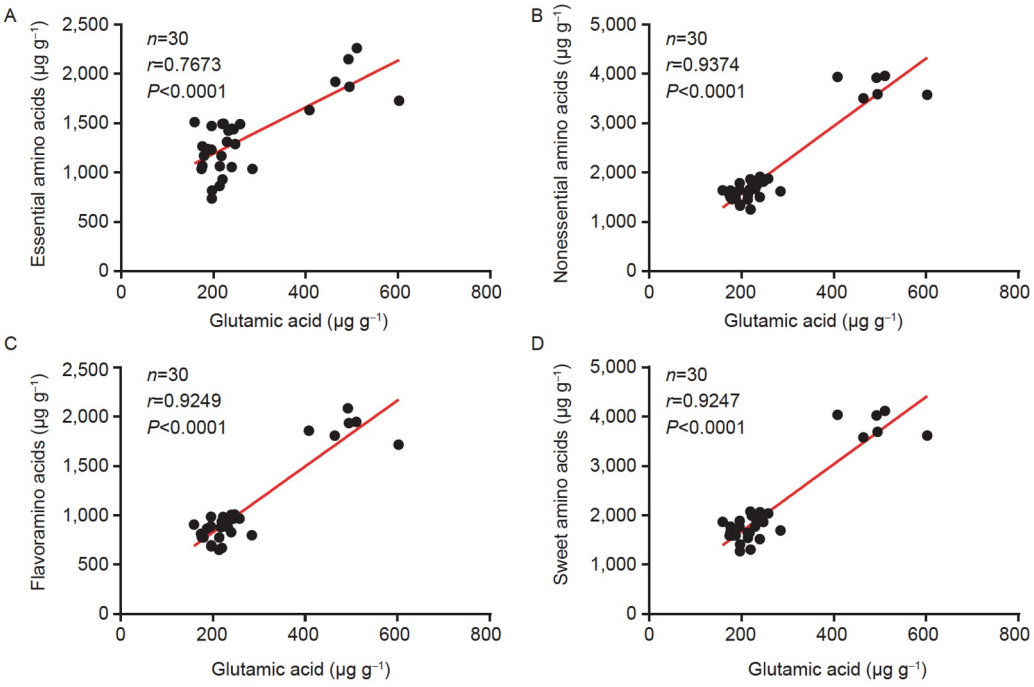

Figure 2 Correlation analyses between the glutamic acid concentration and essential amino acids (A), non-essential amino acids (B), flavor amino acids (C), and sweet amino acids (D) in the *longissimus thoracis* muscle of Shaziling pigs.

(P<0.05). The UFAs content in pork ranged from 57.11% (at 300 days of age) to 60.56% (at 90 days of age). Notably, C16:1 and C18:1n9c were the main MUFAs, and C18:2n6c was the main PUFAs. With increasing age, the percentage of C18:1n9c significantly increased; contents of C16:1 and MUFAs first decreased and then increased; percentage of C18:2n6c significantly decreased; and contents of PUFAs, n3 PUFAs, and n6 PUFAs first increased and then decreased (P<0.05). The turning points of C16:0, C16:1, and C18:0 were observed at 156, 172, and 188 days of age, respectively

Table 1 Amino acid contents in the *longissimus thoracis* muscle of Shaziling pigs at different ages (n=6)

Amino acid contents	30 d (μg g^{-1})	90 d (μg g^{-1})	150 d (μg g^{-1})	210 d (μg g^{-1})	300 d (μg g^{-1})
Nutritionally EAA					
L-Leucine	470.86±31.37^{a}	232.92±22.96^{c}	287.79±21.78^{bc}	343.58±16.83^{b}	341.14±11.77^{b}
L-Isoleucine	274.22±18.70^{a}	97.87±4.22^{d}	147.48±8.83^{c}	205.52±6.13^{b}	178.23±7.54^{b}
L-Valine	137.03±14.54^{a}	87.68±1.54^{b}	88.75±8.28^{b}	154.89±13.52^{a}	148.89±6.15^{a}
L-Histidine	80.05±5.69^{ab}	30.94±0.37^{d}	53.40±7.73^{c}	64.60±6.32^{bc}	87.11±7.59^{a}
L-Lysine	328.89±12.66^{a}	156.86±9.28^{bc}	131.24±14.46^{c}	156.49±8.14^{bc}	167.91±4.65^{b}
L-Methionine	140.37±4.69^{a}	92.20±10.32^{c}	97.36±11.57^{c}	113.95±5.95^{bc}	128.05±4.78^{ab}
L-Phenylalanine	291.31±26.92^{a}	131.44±10.78^{d}	158.58±17.74^{cd}	218.83±4.35^{b}	185.53±6.19^{bc}
L-Threonine	204.31±4.31^{a}	126.86±10.05^{c}	108.43±8.42^{c}	154.80±0.85^{b}	162.88±4.77^{b}
Total EAA	1,927.04±98.58^{a}	956.75±41.27^{c}	1,073.04±72.26^{c}	1,412.65±50.99^{b}	1,399.75±32.96^{b}
Nutritionally NEAA					
L-Alanine	1,563.74±62.49^{a}	549.89±33.62^{b}	630.31±13.54^{b}	630.47±38.35^{b}	662.38±24.08^{b}
L-Arginine	260.65±15.82^{a}	152.42±10.13^{bc}	119.67±20.59^{c}	143.36±8.45^{bc}	168.10±0.22^{b}
L-Aspartic acid	110.37±8.28^{a}	38.70±2.35^{bc}	41.22±2.81^{b}	32.15±3.61^{bc}	26.98±1.96^{c}
L-Glutamic acid	495.55±25.96^{a}	221.59±15.52^{bc}	196.63±6.92^{c}	193.48±10.05^{c}	241.94±4.24^{b}
L-Glycine	798.57±54.71^{a}	251.57±5.35^{b}	287.07±20.88^{b}	331.28±8.48^{b}	299.66±11.08^{b}
L-Serine	280.02±12.24^{a}	112.40±13.61^{c}	124.00±10.25^{c}	172.62±5.23^{b}	176.16±9.15^{b}
L-Tyrosine	208.52±19.17^{a}	119.12±22.00^{c}	154.21±8.77^{bc}	181.15±19.08^{ab}	210.60±3.31^{a}
L-Proline	83.27±8.69^{a}	20.99±3.82^{b}	16.23±1.07^{bc}	5.70±0.95^{c}	27.83±1.86^{b}
L-Cysteine	58.69±2.32^{a}	22.37±3.80^{b}	20.14±2.82^{b}	24.62±0.49^{b}	26.21±3.49^{b}
Total NEAA	3,749.01±86.87^{a}	1,450.33±56.27^{d}	1,548.25±50.04^{cd}	1,682.68±67.80^{bc}	1,812.89±39.14^{b}
Flavor amino acids[1]	1,893.02±52.12^{a}	743.38±32.52^{c}	803.13±30.91^{c}	910.68±31.99^{b}	955.35±21.93^{b}
Sweet amino acids[2]	3,846.91±98.06^{a}	1,528.29±60.36^{c}	1,600.31±75.71^{c}	1,846.36±76.93^{b}	1,927.91±46.03^{b}
Other amino acids					
p-Serine	83.34±6.72^{a}	19.69±3.30^{c}	20.43±1.33^{c}	33.44±0.84^{b}	36.59±6.09^{b}
Taurine	1,068.62±110.34^{a}	502.06±16.72^{b}	512.17±61.02^{b}	594.49±5.12^{b}	475.02±18.24^{b}
L-Citrulline	103.67±5.15^{a}	26.71±2.58^{b}	20.48±1.62^{b}	24.97±3.56^{b}	22.80±1.01^{b}
b-Alanine	183.68±3.04^{a}	202.74±14.86^{a}	125.69±7.10^{b}	90.12±6.20^{c}	79.91±5.84^{c}
L-Ornithine	52.39±2.36^{b}	94.13±12.01^{a}	24.57±0.98^{c}	27.15±1.35^{c}	16.34±0.78^{c}
Anserine	316.15±11.77^{c}	342.52±1.90^{c}	399.11±36.44^{c}	675.82±40.63^{b}	845.42±68.52^{a}
Carnosine	14,870.38±1,091.19^{d}	16,481.15±1,135.01^{d}	20,269.73±1,536.70^{c}	24,350.51±461.03^{b}	27,898.11±481.21^{a}

^{a,b,c,d} Values within a row with different superscripts differ significantly (P<0.05). [1] Flavor amino acids=aspartic acid+Serine+glutamate+Alanine+methionine. [2] Sweet amino acids=Valine+Histidine+Lysine+Threonine+Alanine+arginine+aspartic acid+Glycine+Serine+Proline.

(Figure 3). The SFA/PUFA ratio first decreased and then increased with the increase in age and ranged from 1.44 to 4.14 (P<0.05). With increasing age, the n6/n3 PUFAs ratio, atherogenicity index (AI), and thrombogenicity index (TI) first decreased and then increased, whereas the hypocholesterolemic/hypercholesterolemic (h/H) ratio first increased and then decreased (P<0.05, Table 2). However, no notable quadratic relationships were observed between the age and these parameters (P>0.05, Figure 3). Additionally, C16:0 was negatively correlated with h/H ratio while positively correlated with SFA/PUFA ratio, n6/n3 PUFA ratio, AI, and TI (P<0.05, Figure 4A). In contrast, C18:2n6c was positively correlated with h/H ratio while negatively correlated with SFA/PUFA ratio, n6/n3 PUFA ratio, AI, and TI (P<0.05, Figure 4B). There was no significant linear relationship between C16:1 and the SFA/PUFA ratio (P>0.05), but C16:1 was negatively correlated with h/H ratio while positively correlated with the n6/n3 PUFA ratio, AI, and TI (P<0.05, Figure 4C). No significant linear relationships were observed between C18:0 and the SFA/PUFA ratio, n6/n3 PUFA ratio, or TI (P>0.05); however, C18:0 was positively correlated with h/H ratio while negatively correlated with AI (P<0.05, Figure 4D). There was no significant linear relationship between C18:1n9c and h/H ratio or AI (P>0.05), but C18:1n9c was positively correlated with the SFA/PUFA ratio, n6/n3 PUFA ratio, and TI (P<0.05, Figure 4E).

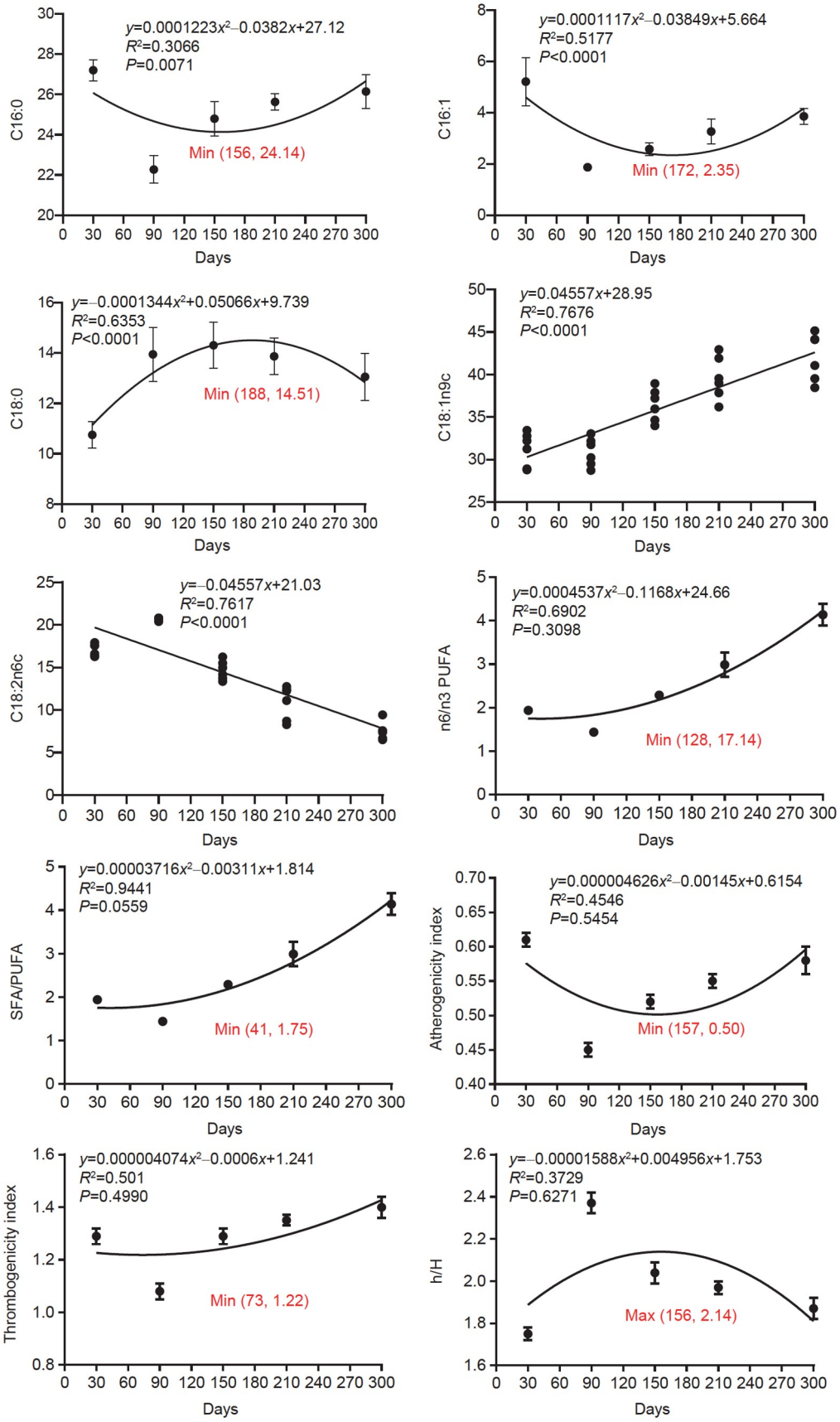

Figure 3 Quadratic regression analysis for the relations between days of age and C16:0, C16:1, C18:0, C18:1n9c, C18:2n6c, SFA/PUFA ratio, n6/n3 PUFAs, h/H ratio, atherogenicity index, and thrombogenicity index in the *longissimus thoracis* muscle of Shaziling pigs.

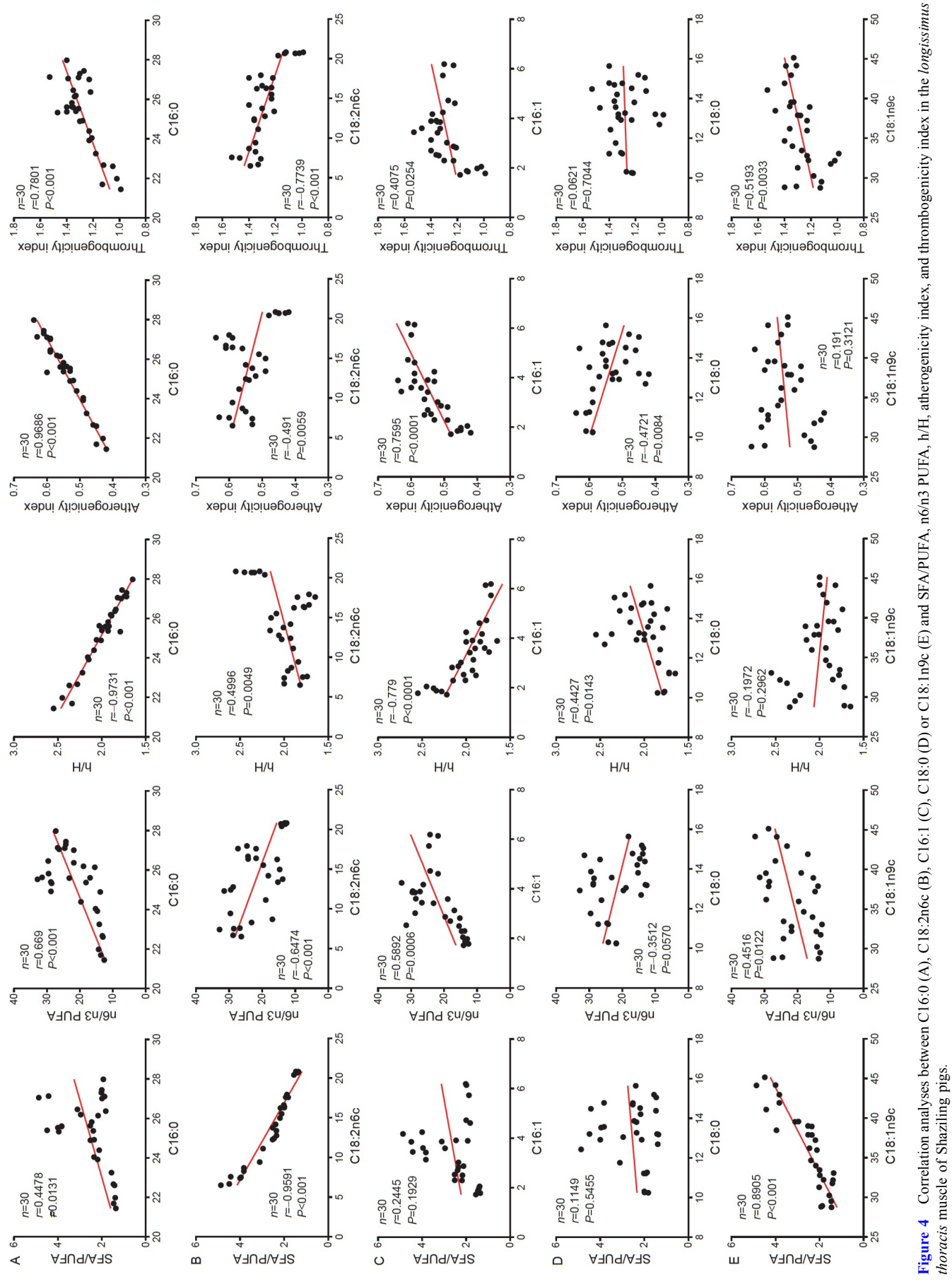

Figure 4 Correlation analyses between C16:0 (A), C18:2n6c (B), C16:1 (C), C18:0 (D) or C18:1n9c (E) and SFA/PUFA, n6/n3 PUFA, h/H, atherogenicity index, and thrombogenicity index in the *longissimus thoracis* muscle of Shaziling pigs.

Table 2 Fatty acid composition in the *longissimus thoracis* muscle of Shaziling pigs at different ages (n=6)

Composition	30 d	90 d	150 d	210 d	300 d
C10:0	0.08±0.01^{d}	0.14±0.01^{c}	0.14±0.01^{c}	0.18±0.01^{b}	0.21±0.01^{a}
C12:0	0.13±0.01^{b}	0.18±0.01^{a}	0.12±0.01^{b}	0.14±0.01^{b}	0.14±0.01^{b}
C14:0	1.93±0.07^{a}	1.08±0.03^{d}	1.32±0.04^{c}	1.51±0.03^{b}	1.62±0.08^{b}
C15:0	0.06±0.00^{b}	0.08±0.01^{a}	0.06±0.01^{b}	0.04±0.01^{c}	0.04±0.01^{c}
C16:0	27.20±0.22^{a}	22.27±0.28^{d}	24.79±0.35^{c}	25.63±0.17^{b}	26.15±0.34^{b}
C16:1	5.21±0.38^{a}	1.88±0.05^{d}	2.58±0.10^{c}	3.27±0.20^{b}	3.86±0.13^{b}
C17:0	0.14±0.00^{b}	0.24±0.01^{a}	0.22±0.01^{a}	0.15±0.00^{b}	0.14±0.01^{b}
C18:0	10.75±0.22^{c}	13.95±0.43^{ab}	14.31±0.37^{a}	13.87±0.30^{ab}	13.05±0.38^{b}
C18:1n9t	0.12±0.00^{bc}	0.11±0.00^{c}	0.11±0.00^{bc}	0.12±0.00^{b}	0.14±0.01^{a}
C18:1n9c	31.24±0.81^{c}	30.91±0.68^{c}	36.45±0.78^{b}	39.58±1.02^{a}	42.08±1.13^{a}
C18:2n6c	17.06±0.29^{b}	20.68±0.06^{a}	14.67±0.45^{c}	10.92±0.80^{d}	7.51±0.42^{e}
C20:0	0.15±0.00^{b}	0.22±0.01^{a}	0.23±0.01^{a}	0.22±0.02^{a}	0.22±0.01^{a}
C18:3n6	0.12±0.01^{a}	0.09±0.00^{b}	0.05±0.00^{c}	0.05±0.00^{c}	0.05±0.01^{c}
C20:1	0.58±0.02^{b}	0.62±0.03^{ab}	0.76±0.06^{a}	0.74±0.06^{a}	0.69±0.02^{ab}
C18:3n3	0.66±0.03^{c}	1.47±0.02^{a}	0.93±0.05^{b}	0.48±0.04^{d}	0.28±0.02^{e}
C20:2	0.54±0.02^{b}	0.70±0.03^{a}	0.55±0.04^{b}	0.34±0.02^{c}	0.27±0.02^{c}
C22:0	0.09±0.00^{b}	0.11±0.01^{a}	0.07±0.00^{cd}	0.08±0.01^{bc}	0.06±0.00^{d}
C20:3n6	0.37±0.03^{b}	0.48±0.01^{a}	0.29±0.02^{b}	0.34±0.05^{b}	0.30±0.04^{b}
C22:1n9	0.14±0.00^{c}	0.22±0.01^{a}	0.16±0.01^{b}	0.10±0.00^{d}	0.07±0.00^{e}
C20:4n6	2.07±0.03^{bc}	3.05±0.03^{a}	1.56±0.17^{c}	2.38±0.40^{b}	1.80±0.18^{bc}
C24:0	0.13±0.01^{b}	0.41±0.01^{a}	0.18±0.03^{b}	0.17±0.03^{b}	0.11±0.01^{b}
C22:6n3	0.16±0.02^{b}	0.34±0.02^{a}	0.11±0.01^{c}	0.08±0.01^{cd}	0.06±0.01^{d}
SFA[1]	40.66±0.34^{a}	38.67±0.60^{b}	41.44±0.62^{a}	41.98±0.34^{a}	41.74±0.56^{a}
UFA[2]	58.27±0.73^{b}	60.56±0.77^{a}	58.21±0.52^{b}	58.41±0.35^{b}	57.11±0.93^{b}
MUFA[3]	37.29±0.96^{c}	33.74±0.71^{d}	40.06±0.81^{c}	43.81±1.04^{b}	46.85±1.22^{a}
PUFA[4]	20.98±0.32^{b}	26.81±0.12^{a}	18.15±0.46^{c}	14.59±1.23^{d}	10.26±0.65^{e}
n3 PUFA[5]	0.82±0.03^{c}	1.81±0.04^{a}	1.03±0.05^{b}	0.56±0.04^{d}	0.34±0.02^{e}
n6 PUFA[6]	19.62±0.32^{b}	24.30±0.08^{a}	16.57±0.46^{c}	13.69±1.22^{d}	9.66±0.62^{e}
SFA/PUFA	1.94±0.03^{c}	1.44±0.03^{d}	2.29±0.06^{c}	2.99±0.28^{b}	4.14±0.25^{a}
n6/n3 PUFA	24.03±0.83^{b}	13.49±0.27^{c}	16.19±0.91^{c}	24.81±2.46^{b}	28.83±0.97^{a}
AI[7]	0.61±0.01^{a}	0.45±0.01^{d}	0.52±0.01^{c}	0.55±0.01^{bc}	0.58±0.02^{ab}
TI[8]	1.29±0.03^{b}	1.08±0.03^{c}	1.29±0.03^{b}	1.35±0.02^{ab}	1.40±0.04^{a}
h/H[9]	1.75±0.03^{d}	2.37±0.05^{a}	2.04±0.05^{b}	1.97±0.03^{bc}	1.87±0.05^{c}

^{a,b,c,d,e} Means with different superscripts within a row differ (P<0.05). [1] SFA=C10:0+C12:0+C14:0+C15:0+ C16:0+C17:0+C18:0+C20:0+C22:0+C24:0, [2] UFA=C16:1+C18:1n9t+C18:1n9c+C18:2n6c+C18:3n6+C20:1+C18:3n3+C20:2+C20:3n6+C22:1n9+C20:4n6+C22:6n3, [3] MUFA=C16:1+C18:1n9t+C18:1n9c+C20:1+C22:1n9, [4] PUFA=C18:2n6c+C18:3n6+C18:3n3+C20:2+C20:3n6+C20:4n6+C22:6n3, [5] n3 PUFA=C18:3n3+C22:6n3, [6] n6 PUFA=C18:2n6c+C18:3n6+C20:3n6+C20:4n6, [7] AI=(4*C14:0+C16:0)/(100−SFA), [8] TI=(C14:0+C16:0+C18:0)/(0.5*MUFA+0.5*∑n6 PUFA+3*n3 PUFA+1/(∑n6:n3 PUFA)), [9] h/H ratio=(C18:1n9t+C18:1n9c+C18:2n6c+C18:3n3+C20:3n6+C20:4n6)/(C14:0+C16:0).

Correlation analyses between meat quality and free amino acid profiles

As shown in Figure 5, the Ans and Car concentrations were positively correlated with pH_{24h}, IMF, and shear force, and negatively correlated with L* and b* values (P<0.05). Contrary to Ans and Car, the concentrations of Asp, β-Ala, and Orn were positively correlated with the L* and b* values, and negatively correlated with pH_{24h}, IMF, and shear force (P<0.05). The concentrations of histidine (His), tyrosine (Tyr), Ile, p-Ser, and FAA were positively correlated with the IMF (P<0.05). The concentrations of Phe, Gly, Ser, FAA, Leu, Ile, EAA, p-Ser, NEAA, SAA, Tau, Cit, Lys, Arg, Cys, Ala, and Glu were negatively correlated with the shear force (P<0.05). The Gly, Ser, FAA, Leu, EAA, p-Ser, Thr, NEAA, SAA, Pro, Lys, Arg, Cys, Ala, and Glu concentrations were positively correlated with the drip loss (P<0.05).

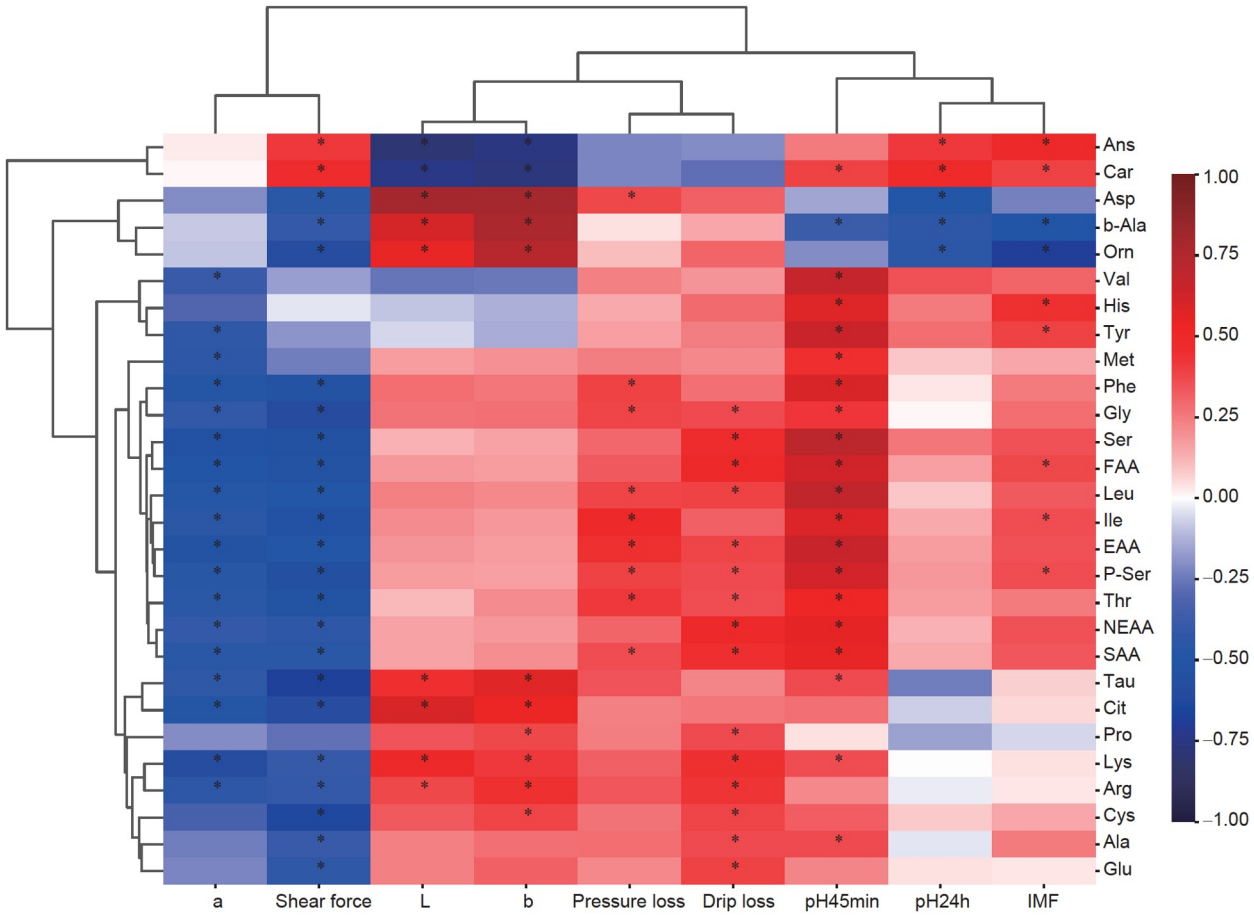

Figure 5 Correlation analysis between amino acid concentrations and meat quality in the *longissimus thoracis* muscle of Shaziling pigs. Different color of blocks denotes different correlation coefficients. Significant correlations between the 2 variables were marked as * for $P<0.05$, ** for $P<0.01$, and *** for $P<0.001$.

Correlation analyses between meat quality and free fatty acid composition

As shown in Figure 6, MUFA, C18:1n9c, and SFA/PUFA ratio were positively correlated with pH_{24h} and IMF, and negatively correlated with the L* and b* values ($P<0.05$). SFA was positively correlated with IMF and negatively correlated with the b* value and drip loss ($P<0.05$). TI was positively correlated with IMF, pH_{45min}, and pH_{24h} ($P<0.05$). The n6/n3 PUFA ratio, C16:1, C16:0, and AI were positively correlated with IMF and pH_{45min} ($P<0.05$). C18:0 was positively correlated with the a* value and shear force and negatively correlated with pressure loss and drip loss ($P<0.05$). The h/H ratio was positively correlated with the a* value and negatively correlated with IMF and pH_{45min} ($P<0.05$). The C22:6n3, C18:2n6c, PUFA, n6 PUFA, C20:2, C18:3n3, and n3 PUFA were negatively correlated with IMF and pH_{24h} and positively correlated with the L* and b* values ($P<0.05$).

Muscle metabolome of Shaziling pigs

A total of 9,075 peaks were detected by UHPLC-MS/MS in the LM of Shaziling pigs. In total, 645 metabolites were identified by comparing with reliable databases. In the following analysis of the muscle metabolome, pigs of different ages are abbreviated as (breed+age), such as S30. The principal component analysis (PCA) revealed that the S30 muscle metabolite patterns were significantly different from those of other Shaziling pigs; the S30, S90, and S210 muscle metabolite patterns were distributed separately, and the muscle metabolite patterns of S90 were significantly different from those of other Shaziling pigs, except S150 (Figure 7A). Orthogonal projection to latent structure-discriminant analysis (OPLS-DA) indicated that there were significant differences in the muscle metabolite patterns between different ages (Figure 7C–F).

Through a Kyoto Encyclopedia of Genes and Genomes (KEGG) database comparison, 106 differential metabolites were enriched in 53 metabolic pathways, including 10 metabolic pathways with *P*-values lower than 0.05 and impact values higher than 0.1, and 72 differential metabolites were enriched in these 10 metabolic pathways. According to KEGG topology analysis, the top two metabolic pathways were Ala, Asp, and glutamate metabolism (impact

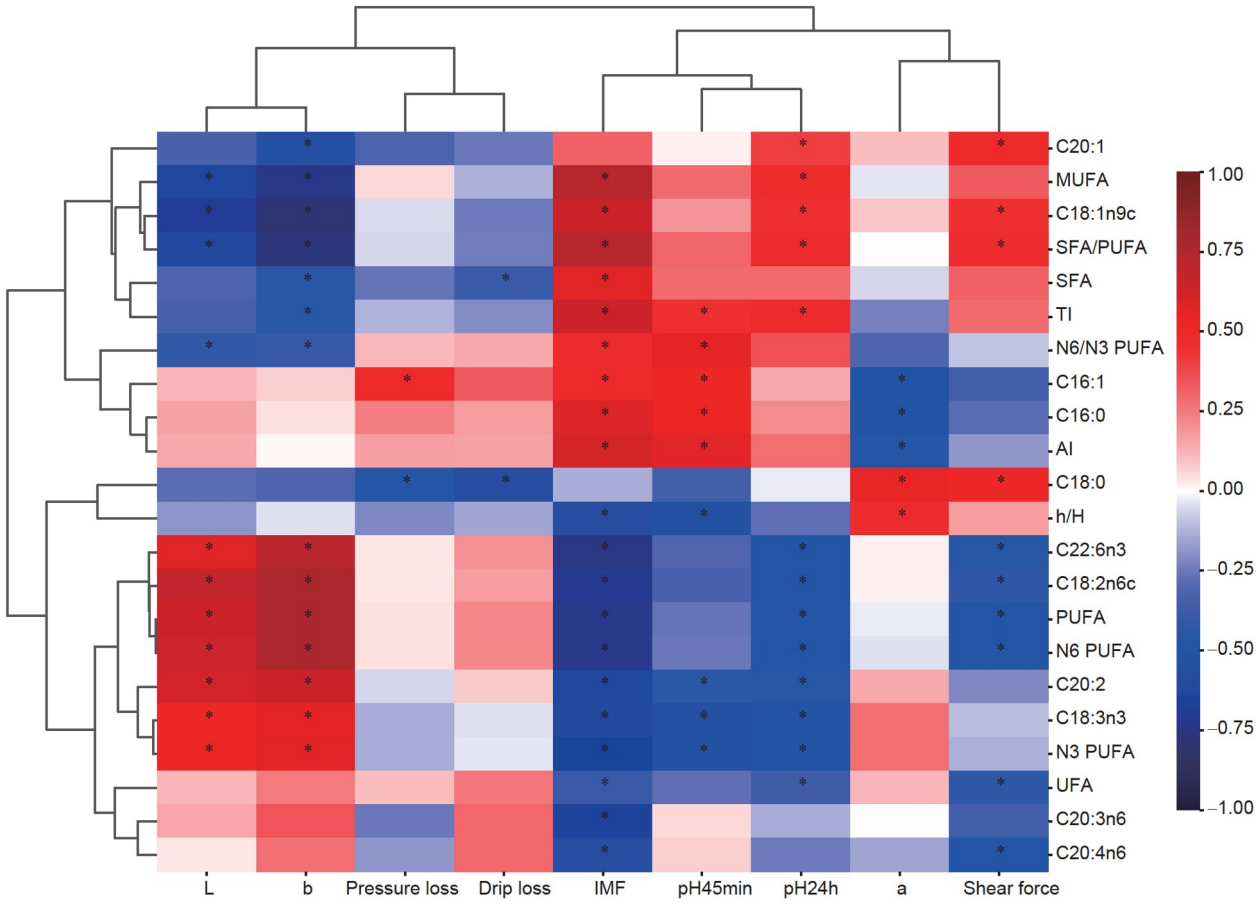

Figure 6 Correlation analysis between fatty acid concentrations and meat quality in the *longissimus thoracis* muscle of Shaziling pigs. Different color of blocks denotes different correlation coefficients. Significant correlations between the 2 variables were marked as * for $P<0.05$, ** for $P<0.01$, and *** for $P<0.001$.

value=0.66, $P<0.01$) as well as D-glutamine and D-glutamate metabolism (impact value=0.62, $P<0.001$). The top three abundant metabolites enriched in these metabolic pathways were Glu, Asp, and L-glutamine. Basic information is shown in Table S2 in Supporting Information, and overview of the differential metabolic pathways is shown in Figure 8A. Based on the KEGG pathway database, the main pathways of LM meat metabolism influenced by age are summarized in Figure 8B.

To identify the most relevant metabolic pathways involved in the metabolic response of Shaziling pigs of different ages, we submitted the differential metabolites to the KEGG website for the analysis of relevant pathways. As shown in Figure 8C, the KEGG enrichment analysis revealed that the following pathways of the first 20 abundances were significantly changed in the LM of Shaziling pigs: protein digestion and absorption:OS, central carbon metabolism in cancer:HD, β-Ala metabolism:M, His metabolism:M, aminoacyl-tRNA biosynthesis:GIP, purine metabolism:M, ABC transporters:EIP, choline metabolism in cancer:HD, pantothenate and CoA biosynthesis:M, FoxO signaling pathway: EIP, arginine biosynthesis:M, glycerophospholipid metabolism:M, cocaine addiction:HD, Ala, Asp, and Glu metabolism:M, mineral absorption:OS, glutamatergic synthase:OS, Phe metabolism:M, amphetamine addiction:HD, GABAergic synapse:OS, and retrograde endocannabinoid signaling: OS.

Correlation analyses between meat quality and muscle metabolites

To identify potential meat quality indicators, correlation analyses were performed between the top 30 metabolites and the meat quality-related parameters. As shown in Figure 9, glutathione, pantothenic acid, D-pantothenic acid, and Glu were positively correlated with the L* and a* values and negatively correlated with pH_{45min} and/or pH_{24h} ($P<0.05$). In contrast, His, Ans, and histamine were negatively correlated with the L* and a* values and positively correlated with pH_{45min} and/or pH_{24h} ($P<0.05$). Pressure loss was positively correlated with LPC(18:1) but negatively correlated with His, D-erythrose-4-phosphate, Ans, and histamine ($P<0.05$). Shear force was positively correlated with His, betaine, Ans, histamine, and PC[15:0/18:2(9Z,12Z)] but negatively

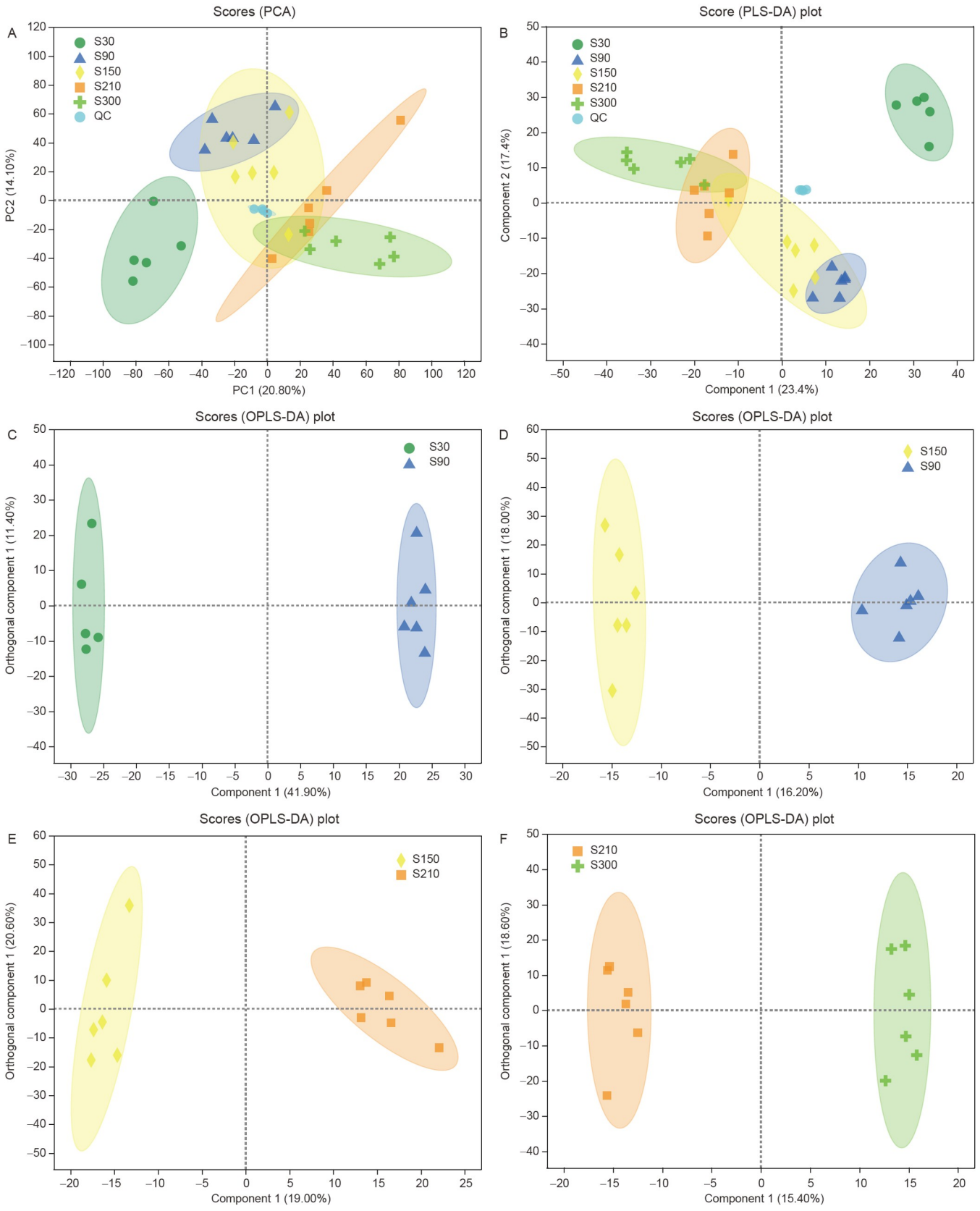

Figure 7 PCA, PLS-DA, and OPLS-DA scatter plots of Shaziling pigs at different ages. (A) PCA of pigs, (B), PLS-DA of pigs, (C–F) OPLS-DA scatter plots of pigs at different ages. PLS-DA, partial least squares discrimination analysis. Pigs of different ages were abbreviated as (breed+age), such as S30 means Shaziling pigs at 30 d of age.

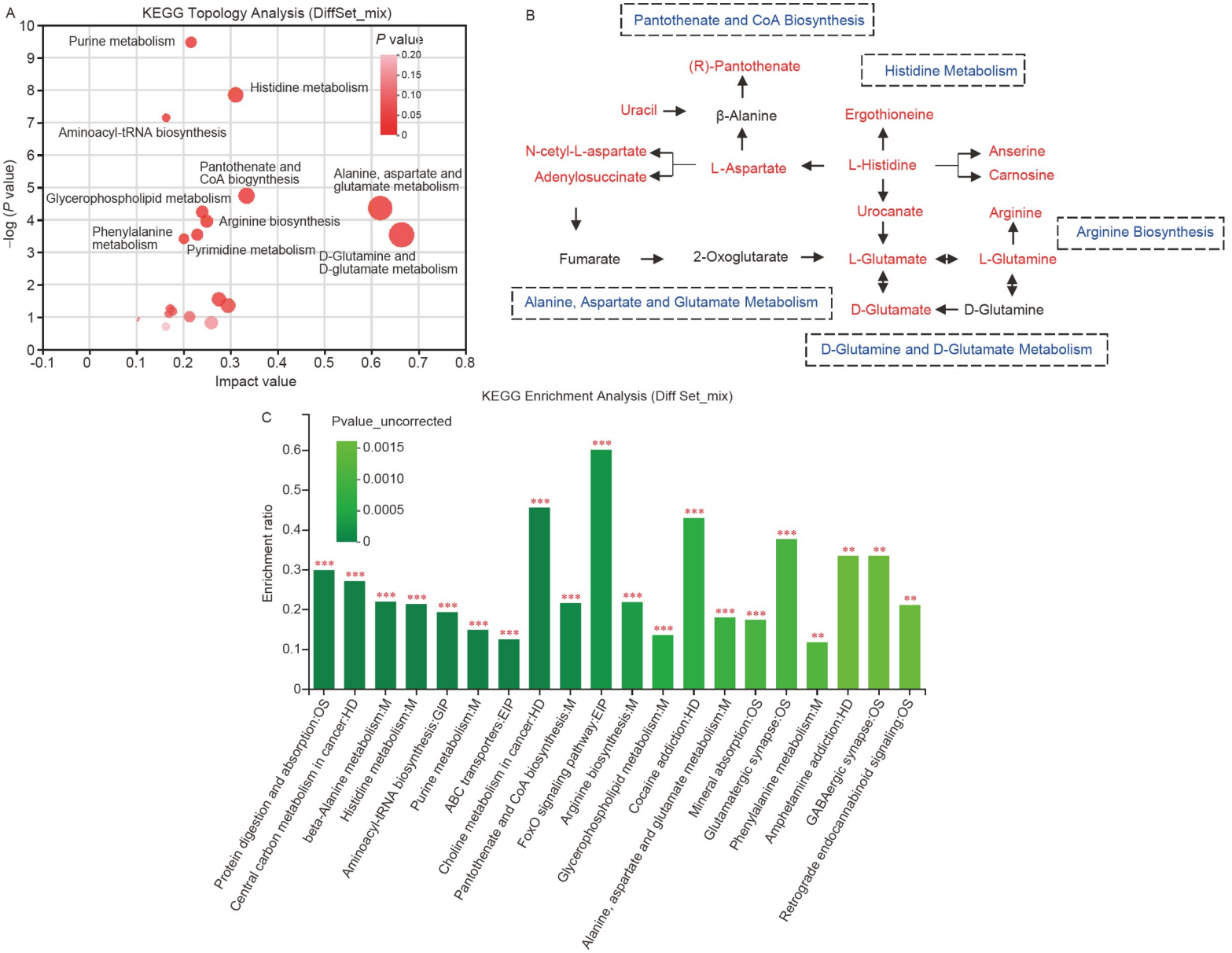

Figure 8 Metabolic pathway analysis by using the UHPLC-MS/MS. A, Overview of pathway analysis offers a metabolomics view, which displays all matched pathways as circles. The color and size of each circle is based on the *P* value and the pathways impact value, respectively. B, Metabolic pathways affected by Shaziling pigs with different age. Metabolites colored in red refers to the metabolite were found from the Shaziling pig meat. Metabolites in black were not detected. C, Metabolic pathway enrichment analysis. OS, organismal systems; HD, human diseases; M, metabolism; GIP, genetic information processing; EIP, environmental information processing; CP, cellular process. These were added as suggested (* for $P<0.05$, ** for $P<0.01$, and *** for $P<0.001$).

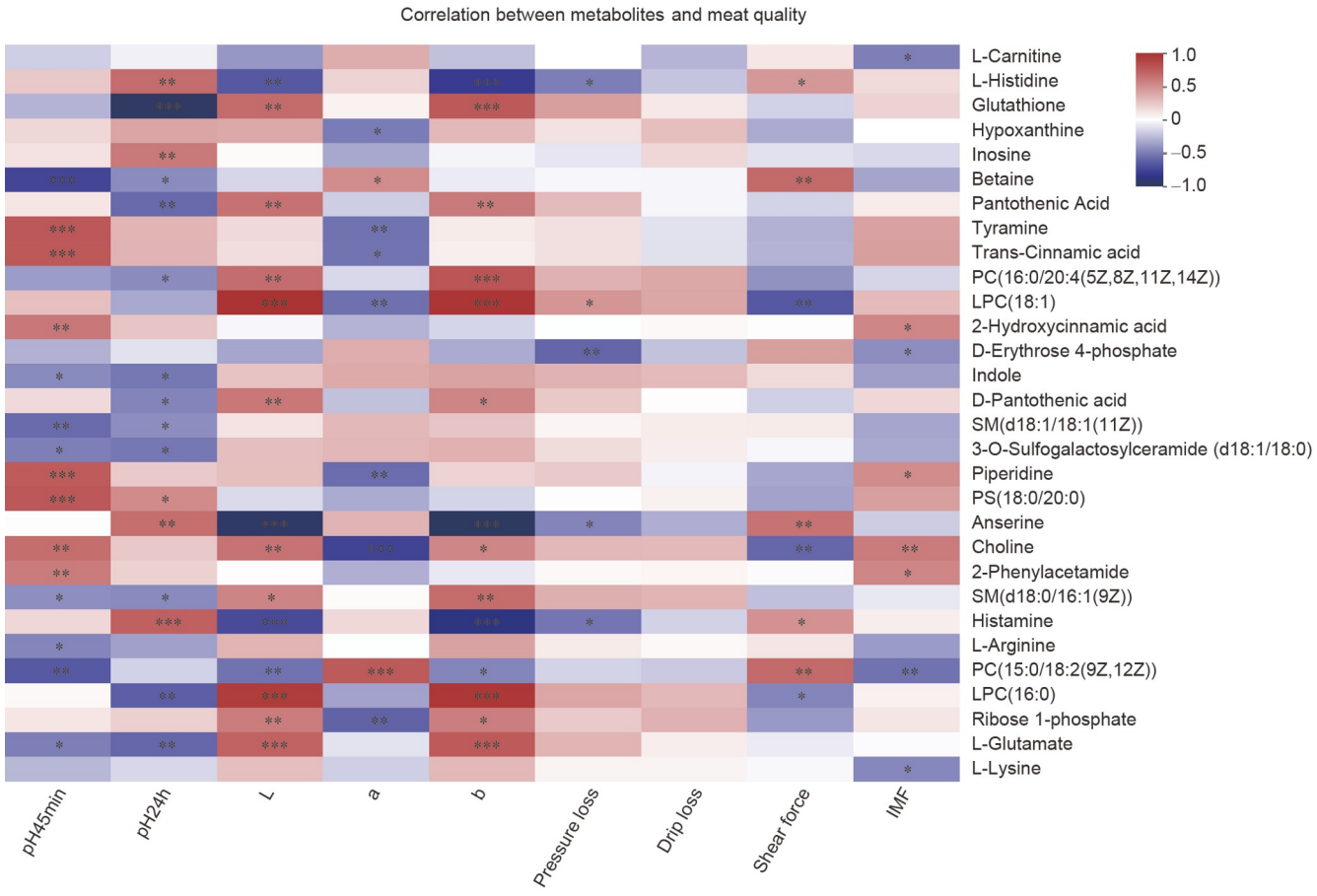

Figure 9 Correlation analysis between meat quality and the top 30 metabolites in the *longissimus thoracis* muscle of Shaziling pigs. Different color of blocks denotes different correlation coefficients. Significant correlations between the 2 variables were marked as * for $P<0.05$, ** for $P<0.01$, and *** for $P<0.001$.

correlated with LPC(18:1), choline, and LPC(16:0) ($P<0.05$). There was a positive correlation between IMF and 2-hydroxycinnamic acid, piperidine, choline, and 2-phenylacetamide and a negative correlation between IMF and Car, D-erythrose-4-phosphate, PC[15:0/18:2(9Z,12Z)], and Lys ($P<0.05$).

DISCUSSION

The Shaziling pig is a typical small pig breed in China with good meat quality. However, no information has been reported on the basic biological characteristics of the meat quality of Shaziling pigs. Extensive research has ascribed age and weight as the major factors affecting meat quality (Čandek-Potokar et al., 1998; Lo Fiego et al., 2010). Here, the metabolites, amino acid and fatty acid composition of the LM of Shaziling pigs were analyzed at different ages, and the correlation of these parameters with meat quality traits was also determined.

As a complex trait, meat quality can be comprehensively assessed using a series of indicators, such as pH, meat color, drip loss, IMF, and shear force (Schwab et al., 2006). Drip loss is associated with the water-holding capacity of meat, shear force can reflect meat tenderness, and IMF is strongly related to the flavor, juiciness, and tenderness of meat (Toldrá, 2003; Zhang et al., 2021b). Previous studies using poultry models reported that the water-holding capacity and IMF content of meat increased with age (He et al., 2018; Li et al., 2021). In line with these findings, our data showed that age significantly affected the drip loss, shear force, and IMF content of LM meat from Shaziling pigs. The current study also found that the age-dependent values of pH_{45min} and pH_{24h} increased, whereas the opposite trend was observed for L*. The pH value analyzed 45 min post mortem is a pork quality predictor and is often used to classify meat for further processing at the slaughter line (Kusec et al., 2003). Meat with a pH_{45min} value of 5.8 or less is regarded as pale soft exudative (PSE) meat, and a value between 5.8 and 6.0 is viewed as suspicious and above 6.0 is normal (Hoffman, 1994). The pH value analyzed 24 h post mortem can reflect whether the meat is consumed fresh or used as a raw material for processed meat products. Its value within the range of 5.5–5.7 exhibits the most desirable quality attributes, and

moving away from this range in either direction is detrimental to quality (Matarneh et al., 2021). Our analyses indicated that the pH_{45min} value was above 6.0, and the pH_{24h} value was within the range of 5.5–5.7 in the LM of Shaziling pigs at each age. These findings suggest that age greatly influences the sensorial and nutritional quality of Shaziling pig meat, and the meat color and storage quality improve with age.

Amino acids can affect the taste and flavor of meat by providing nutritive value and flavor characteristics (Guo et al., 2020; Ma et al., 2020). Classically, amino acids function as building blocks of protein, and are divided into EAAs (indispensable) and NEAAs (dispensable), based on the needs of the diet for nitrogen balance or growth (Wu, 2009). In the current study, both EAA and NEAA contents first decreased and then increased with increasing age, and their turning points were 178 and 200 days, respectively. Therefore, it is posited that NEAA, rather than EAA, plays key roles in muscle growth and development from 178 to 200 days of age. Amino acids can also be classified as SAAs and FAAs (Gao et al., 2018). We found that with increasing age, the content of SAAs and FAAs first decreased and then increased, and the turning point was 198 days. These findings are in contrast to the results observed in Liangshan pigs (Gan et al., 2020), in which the contents of these amino acids were relatively stable at different bodyweights (53.2, 59.5, 67.4, 74.9, 80.4, 86.7, and 91.5 kg), but EAA content showed an upward trend. The inconsistency in the above reports might be attributable to differences in breed and slaughter age. Interestingly, further analysis revealed a significantly positive correlation between muscle Glu concentrations and the EAA, NEAA, FAA, and SAA contents. These observations raise the possibility that muscular Glu concentration could be used as a biomarker to predict the EAA, NEAA, FAA, and SAA contents in Shaziling pigs. Next, we also found that Glu concentration was positively correlated with drip loss and negatively correlated with shear force, suggesting that increasing Glu content would decrease the water-holding capacity but increase the meat tenderness in Shaziling pigs. Additionally, Pearson correlation analysis revealed that increasing the Ans and Car concentrations could increase the IMF contents and pH_{24h} and decrease the L* and b* values, thus improving the juiciness and color of meat. In contrast, Asp, β-Ala, and Orn were negatively correlated with meat quality traits.

Fatty acids are of great importance to the nutritional value of meat and are closely related to its eating quality (Wood et al., 2008; Zhang et al., 2019). In the current study, the predominant SFAs identified in the LM of Shaziling pigs were C16:0 and C18:0, which is in accordance with several published reports (Choi et al., 2016). Moreover, we found that C18:0 and SFA contents were negatively correlated with drip loss, while C16:0 and SFA contents were positively correlated with IMF. Other studies have corroborated these results (Suzuki et al., 2006; Zhang et al., 2019). These findings indicate that increasing C18:0 would help to increase the water-holding capacity, whereas increasing C16:0 might increase meat juiciness in Shaziling pigs. The most dominant MUFAs identified in the LM of Shaziling pigs were C18:1n9c, followed by C16:1. These results differ from those of other studies (Tu et al., 2021), which reported that the main MUFAs in the pork of Dongbei Min pigs or Wuzhishan pigs were C18:1n9t and C20:1. An important reason for the different observations may be the difference in breeds. Additionally, a positive correlation was observed between MUFA (especially C16:1 and C18:1n9c) content and IMF or pH_{24h}, whereas a negative correlation was observed between MUFA content (especially C18:1n9c) and the values of L* and b*. In parallel with these positive correlations between MUFAs and meat quality, previous studies have reported a positive correlation between MUFAs (C16:1, C18:1) and pork flavor, flavor preference, and overall acceptability (Suzuki et al., 2006). These observations suggest that increasing MUFA contents (especially C16:1 and C18:1n9c) in the LM of Shaziling pigs would help improve meat quality traits. In contrast to SFAs and MUFAs in the LM of pigs, PUFAs are negatively correlated with pork flavor and the overall meat acceptability (Cameron and Enser, 1991; Cameron et al., 2000). In this study, the major PUFA identified in the LM of Shaziling pigs was C18:2n6c. Both C18:2n6c and PUFA contents were positively correlated with the values of L* and b* and inversely proportional to IMF and pH_{24h}. Other studies corroborate these results (Ros-Freixedes and Estany, 2014). Therefore, in terms of nutritional value, replacing PUFAs (C18:2n6c) with SFAs (C16:0, C18:0) and MUFAs (C16:1) would help produce more nutritious pork.

Moreover, this study found that C16:0 was positively correlated with the SFA/PUFA ratio, n6/n3 PUFA ratio, AI, and TI and negatively correlated with the h/H ratio. However, the opposite trend was observed for the correlation between C18:2n6c and these parameters. The effects of fatty acid composition on cardiovascular diseases can be assessed using the h/H ratio, AI, and TI indices. Specifically, a relatively low AI and TI content and a high h/H ratio lead to a lower incidence of cardiovascular disease (Chen and Liu, 2020). These findings suggest that C16:0 and C18:2n6c could be potential indicators of the health attributes of fatty acid composition in the LM of Shaziling pigs. From a health perspective, reducing C16:0 content and simultaneously elevating C18:2n6c in pork would be beneficial to human health. Moreover, the aged Shaziling pigs (150, 210, and 300 days of age) were characterized by a significantly lower PUFA content (C18:2n6c) and a significantly higher MUFA content (C18:1n9c) than those of the young Shaziling pigs (30 and 60 days of age). These findings indicate that, from the perspective of fatty acids, aged Shaziling pigs are heal-

thier, safer, nutritious, and high-quality meat. Additionally, these predominant fatty acids identified in the LM of Shaziling pigs (C16:0, C16:1, C18:0, C18:1n9c, and C18:2n6c) made up about 89.69%–93.27% of the total fatty acids, suggesting that these fatty acids may be the material basis for the excellent meat quality of Shaziling pigs.

Metabolomics is applied for the investigation of key metabolites contributing to physico-chemical properties, and hence, it helps to account for meat quality traits (Muroya et al., 2020). Metabolic pathway analysis has allowed us to better understand the direct internal connections of metabolites, which can reconstruct biochemical reaction networks (Klamt and Stelling, 2003). KEGG pathway enrichment analysis revealed that Ala, Asp, and Glu, D-Glutamine, and D-glutamate metabolism; pantothenate and CoA biosynthesis; histidine metabolism; arginine biosynthesis; glycerophospholipid, pyrimidine, purine, and Phe metabolism; and aminoacyl-tRNA biosynthesis were the significant LM meat metabolic pathways affected by age. To the best of our knowledge, this is the first study to analyze the muscular metabolome of Shaziling pigs. However, contradictory results have been reported for chicken breast muscles, in which fructose and mannose metabolism, arachidonic acid metabolism, steroid hormone biosynthesis, riboflavin metabolism, biosynthesis of UFAs, and linoleic acid metabolism were significant metabolic pathways influenced by age (Li et al., 2022). Additionally, we found that the top three abundant metabolites enriched in these pathways were Glu, Asp, and L-glutamine. Our current results differ from those of Wang et al. (2020), where lactate, creatine, and Ans were the three most abundant metabolites in duck breast meat. The inconsistency of the above reports might be attributable to the differences in the experimental breed and age used in each experiment. Furthermore, Pearson correlation analysis revealed that Glu was positively correlated with L* and b* values and negatively correlated with pH_{45min} and pH_{24h}. These findings indicated that apart from water-holding capacity and tenderness, Glu also affected meat color and post-mortem pH. Apart from Glu, the other four metabolites, including glutathione, LPC(18:1), Ans, and LPC(16:0), were also strongly related to the quality traits of LM meat in Shaziling pigs. Considering the results obtained from the analysis of the correlation between amino acid composition and meat quality, we conclude that Ans might be regarded as a predictor of meat quality in the LM of Shaziling pigs. Although mathematical correlations between these metabolites and meat quality traits were established in this study, the biological relationship between them requires further evaluation.

CONCLUSIONS

The results of our research highlighted that age plays an important role in meat quality, amino acid and fatty acid composition, and metabolite profiles of LM in Shaziling pigs. Muscular Glu concentrations could be used as biomarkers to predict the EAA, NEAA, FAA, and SAA contents in Shaziling pigs. The predominant fatty acids identified in the LM of Shaziling pigs were C16:0, C16:1, C18:0, C18:1n9c, and C18:2n6c. Ala, Asp, Glu, D-glutamine, and D-glutamate metabolism were the main metabolic pathways for the Shaziling pig meat flavor. Given its highly positive correlation with IMF and/or pH_{24h} and negative correlation with the values of L* and b*, the muscular contents of Ans, C16:0, C16:1, and C18:1n9c might be promising indicators of better meat quality. These findings may contribute to the improvement of Shaziling pig meat quality at the genetic and molecular levels. Further studies are needed to explore the potential molecular mechanisms of Shaziling pig meat to determine the factors that cause these age-related differences in meat quality in the current study.

MATERIALS AND METHODS

Animals and sample collection

All experiments described in this study were performed according to the guidelines for animal experiments released by the Animal Care Committee of the Institute of Subtropical Agriculture, Chinese Academy of Sciences (License No.: ISA-2020-023).

Thirty healthy male Shaziling pigs were selected and raised under the same environmental conditions with the same feed and water available at all times. Six pigs were slaughtered at different slaughtering ages (d30, d90, d150, d210, and d300). After slaughter, LM samples were rapidly cut between the 9th and 10th ribs from the right half of each pig, frozen in liquid nitrogen, and preserved at −80°C for UHPLC-MS/MS analysis. Subsequently, the LM on the left half of each pig between the 10th and 12th ribs was sampled for the analysis of meat quality and amino acid and fatty acid contents.

Meat quality

The pH values were determined using a portable pH meter (Matthaus pH Star, Germany) at 45 min and 24 h post mortem.

The color parameters were measured using a chromameter (CR-410, Kinica Minolta Sensing Inc., Japan) at two different locations and are described as CIE L* (lightness), a* (redness), and b* (yellowness).

Drip loss and IMF content were measured following the methods described by Song et al. (2022). Briefly, to measure the drip loss, LM samples were cut into cubes (1 cm×1× cm 2 cm), weighed, and suspended in plastic cups for 24 h at

4°C. The percentage of weight loss to the initial weight was recorded as drip loss. The IMF content was determined using petroleum ether and a Soxtec extraction method.

To measure shear force, fresh LM samples were cooked at 80°C to an internal temperature of 70°C. After cooling, the cooked samples were cut into cuboids (2 cm×1 cm×1 cm) in the direction of the myofibrils and then cut perpendicular to the fiber orientation of the muscle. Shear force values were determined using a Warner-Bratzler shear force device (TA. XT Plus, Stable Micro Systems, UK), as previously described (Li et al., 2018). Each sample was replicated eight times.

The free amino acid profile

The free amino acid profile was measured as previously described in our work (Li et al., 2015). Briefly, freeze-dried samples of LM (0.5 g) and hydrochloric acid (5 mL, 0.01 mol L^{-1}) were homogenized for 30 min and centrifuged at 10,000×*g* for 15 min. The supernatant (2 mL) was homogenized with n-hexane (2 mL). After demixing, the lower tier (1 mL) and 0.8% sulfosalicylic acid solution (1 mL) were mixed and incubated at room temperature for 15 min followed by centrifugation at 10,000×*g* for 10 min. The supernatant was then filtered through a 0.2-μm filter. The filtrate was labeled with iTRAQ reagents (AA 45/32 kit; Applied Biosystems, USA) as recommended by the manufacturer and analyzed using high-performance liquid chromatography (1260 Infinity II; Agilent Technologies Inc., USA). The sum of FAA was calculated as the sum of the Asp, Ser, Glu, Ala, and Met contents. The sum of SAA was calculated as the sum of the Asp, Ser, Gly, Ala, His, Arg, Pro, Val, Thr, and Lys contents.

Fatty acid composition

The fatty acid composition was measured as previously described in our work (Zhang et al., 2022). Briefly, 150 mg lyophilized muscle samples were analyzed by gas chromatography (HP 6890 series, Hewlett Packard, USA) using a DB-23 capillary column (length, 60 m; internal diameter, 0.25 mm; film thickness, 0.25 μm; Agilent Technologies Inc.) and a flame ionization detector.

The fatty acid composition was expressed as a percentage of the total fatty acid content. The sum of SFAs was calculated as the sum of the percentages of capric acid (C10:0), lauric acid/dodecanoic acid (C12:0), myristic acid (C14:0), ginkgolic acid (C15:0), palmitic acid (C16:0), heptadecanoic acid (C17:0), stearic acid (C18:0), eicosanoic acid/arachidic acid (C20:0), and docosanoic acid (behenic acid) (C22:0). The sum of UFAs was calculated as the sum of the percentages of palmitoleic acid (C16:1), methyl trans oleate/trans-9-octadecaenoic acid methyl ester (C18:1n9t), methyl oleate/cis-9-octadecaenoic acid methyl ester (C18:1n9c), methyl linoleate/cis, cis-9,12-octadecadienoic acid methyl ester (C18:2n6c), γ-methyl linolenate (C18:3n6), eicosenoic acid (C20:1), α-methyl linolenate (C18:3n3), arachidonic acid (C20:2), cis 8,11,14-eicosotrienic acid methyl ester (C20:3n6), cis erucic acid methyl ester (C22:1n9), arachidonic acid methyl ester (C20:4n6), and docosahexaenoic acid (C22:6n3). The sum of the MUFAs was calculated as the sum of the percentages of C16:1, C18:1n9t, C18:1n9c, C20:1, and C22:1n9. The sum of PUFAs was calculated as the sum of the percentages of C18:2n6c, C18:3n6, C18:3n3, C20:2, C20:3n6, C20:4n6, and C22:6n3. The SFA:PUFA ratio was calculated by dividing the sum of SFA by the sum of PUFA. The sum of all n6 PUFAs (C18:2n6c, C18:3n6, C20:3n6, and C20:4n6) was divided by the sum of all n3 PUFAs (C18:3n3 and C22:6n3) to calculate the n6/n3 PUFA ratio. Lipid quality indices such as the h/H, AI, and TI were calculated as previously described (Zheng et al., 2021).

Sample extraction and UHPLC-MS/MS analysis

Each sample from the LM (50 mg) was extracted in 400 μL of aqueous methanol solution (CH_3OH:H_2O=4:1) in a 2.0 mL Eppendorf tube. Samples were homogenized for 6 min using a TissueLyser (−10°C, 50 Hz) and discontinuous ultrasonication for 30 min on ice (5°C, 40 kHz), and then incubated at −20°C for 30 min. After centrifugation at 13,000×*g* for 15 min at 4°C, the supernatants were collected and transferred to new Eppendorf tubes for UHPLC-MS/MS analysis, as described in our previous study (Song et al., 2022).

Data analysis

After the UHPLC-QE-HFX/MS analyses, the raw data were imported into the metabonomics processing software Progenesis QI (Waters Corporation, USA) for data preprocessing, such as baseline filtering, peak identification, integration, retention time correction, and peak alignment. The pretreated metabonomics data were analyzed using PCA and OPLS-DA using the ropls (Version1.6.2, http://bioconductor.org/packages/release/bioc/html/ropls.html) R package on the Majorbio Cloud Platform (https://cloud.majorbio.com). PCA was conducted to detect intrinsic clusters and outliers within the dataset. OPLS-DA was performed to compare the metabolite differences between the different ages of haziling pigs. A *t*-test (Student's *t*-test) combined with the multivariate OPLS-DA analysis was used to evaluate the differential metabolites or potential markers (variable importance in the projection (VIP)>1, $P<0.05$) to reveal the differences in the metabolic composition and pathway among groups.

Meat quality and composition of amino acids and fatty

acids at different ages are expressed as mean±SEM and analyzed by one-way ANOVA using SAS 8.2. When the data did not comply with the normal distribution or homogeneity, significance was determined using the Kruskal-Wallis test. Different lowercase letters indicate significant differences at different ages (P<0.05).

Linear and quadratic regression analyses were performed for meat quality and the composition of amino acids and fatty acids using GraphPad Prism 7.04 and PROC REG of SAS software, respectively.

The correlational heatmaps were generated according to the results of Pearson correlation analysis, which was conducted in the environment of the Python package Scipy.stats (https://docs.scipy.org/doc/scipy/). Significant differences are marked as * for P<0.05, ** for P<0.01, and *** for P<0.01.

Compliance and ethics *The author(s) declare that they have no conflict of interest.*

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