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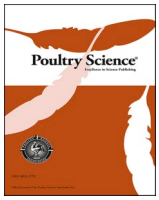

Network pharmacology and molecular docking reveal the mechanism of Chinese herb ultrafine powder improving meat nutritional value in aged laying hens

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ABSTRACT

This study investigated the effects of dietary Chinese herb ultrafine powder (**CHUP**) supplementation on meat quality, plasma biochemical parameters, and fatty acid and amino acid composition in pectoral muscles of aged laying hens. A total of 576 Xinyang black-feather laying hens (300-d-old) were randomly allocated to eight groups, including the control group (fed a basal diet) and different CHUP groups (details in 'Materials and methods' section). The trial lasted 120 d. The findings showed that L-LF and L-LF-T supplementation increased the contents of polyunsaturated fatty acids and unsaturated fatty acids ($P < 0.05$), while CHUP supplementation increased ($P < 0.05$) the total essential amino acid content in pectoral muscles. Network pharmacology analysis predicted that L-LF-T supplementation mainly influenced the PPAR signaling pathway, which is associated with meat quality. These findings suggest that CHUP supplementation can enhance the nutritional value of pectoral muscles, potentially through its association with the PPAR signaling pathway in aged laying hens.

Introduction

Poultry meat is well known for its high protein content and as a substantial source of vitamins, minerals, and other vital nutrients that are either exclusive or more bioavailable in animal-derived foods (Leroy and Cofnas, 2020). Poultry is an excellent protein source for human nutrition due to its rapid growth rates and substantial yields (Escobedo Del Bosque et al., 2022). Chicken meat, in particular, is distinguished by its high content of polyunsaturated fatty acids (**PUFA**), a characteristic attributed to the high PUFA levels in broiler diets (Smet et al., 2008). In the context of poultry production, aged laying hens are frequently considered discardable byproducts, with meat that is often deemed to have lower functional quality, higher fat and cholesterol levels, and an overall decline in palatability and market value (Kumar et al., 2023). However, a substantial portion of the revenue generated from egg-laying hen farming is derived from the sale of these culled hens. For instance, approximately 20 % of the income from laying hen farming comes from culled hens, which contribute over 1.5 million metric tons of low-value meat annually in China (Ren et al., 2016). The toughness of meat for aged laying hens is influenced by several factors, including elevated collagen content, reduced proteolytic enzyme activity, and muscle fiber degradation (Katemala et al., 2021). These factors collectively contribute to increased shear force and reduced tenderness. Therefore, implementing strategic nutritional interventions to enhance the meat quality of late-stage laying hens could not only enhance economic returns but also promote the sustainable development of the industry.

It has been found that the Chinese herbs and their bioactive compounds supplementation in chicken diets can enhance the meat quality of broiler chickens by modifying the fatty acid profile (Jachimowicz et al., 2022). Numerous prior studies have demonstrated the advantages of utilizing herbs as feed additives for improving the meat quality of broilers. For instance, the supplementation of dandelion has been found

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to increase the a* value, decrease the cooking loss, and elevate the proportion of PUFA in the *pectoralis major* muscle of Arbour Acres broiler chickens (Wang et al., 2023). Additionally, Wu et al. (2023) suggested that dietary supplementation with *Anoectochilus roxburghii* extract is a viable option for a novel feed additive that may reduce abdominal fat deposition and enhance the meat quality in broilers. Moreover, *Litsea cubeba* fruit extract supplementation has been shown to enhance the taste and nutritional value of White-feather broiler meat (Luo et al., 2024). Our previous studies have also indicated that the Chinese herb ultrafine powder (**CHUP**) can improve the nutritional value of eggs (Gui et al., 2024) and promote the proliferation of beneficial bacteria (including *Blautia, Carnobacterium*, and *Clostridiales*) in the jejunum (Gui et al., 2023) of late-stage laying aged hens. However, considering that CHUP is a composite formulation comprising three Chinese medicinal herbs, further research is necessary to comprehensively elucidate its essential bioactive constituents and potential mechanisms.

Network pharmacology is based on the interaction networks between drugs, genes, targets, and diseases, systematically elucidating the effects of pharmacological agents on various diseases (Zhang et al., 2019). Currently, there are numerous studies focusing on the influence of Chinese herbs on meat quality in broilers; however, investigations into the influence of CHUP on meat quality in aged laying hens remain scarce. Therefore, this study hypothesized that CHUP may enhance the meat quality and nutritional value of aged laying hens. To test this hypothesis, the present study employed an integrated methodology that combines network pharmacology and molecular docking for the first time with experimental approaches to construct a Chinese herb-meat quality interaction network to elucidate the key bioactive components, core target genes, and potential pathways through which CHUP influences the meat quality and nutritional value of Xinyang black-feather aged laying hens, thereby bridging the use of traditional herbs with modern computational biology. Furthermore, the findings of this investigation will provide a theoretical foundation for the effective utilization of resources from aged laying hens.

Materials and methods

Animal ethics statement

The animal study was reviewed and approved by the Animal Care and Use Committee of the Institute of Subtropical Agriculture, Chinese Academy of Sciences, Changsha, China (ISA-2018-071).

Experimental birds, diets, and management

A total of 576 Xinyang black-feather laying hens, at 300-d-old (at the beginning of the late laying phase) with uniform egg-laying capabilities, were randomly assigned to eight experimental groups. Each group consisted of eight replicates, with nine laying hens in each replicate. The control group was fed a basal diet, while the treatment groups received the basal diet supplemented with different CHUP additives: 0.5 % *Leonuri herba* (**L group**), 0.25 % *Ligustri lucidi fructus* (**LF group**), 0.25 % *Taraxaci herba* (**T group**), 0.5 % L + 0.25 % LF (**L-LF group**), 0.5 % L + 0.25 % T (**L-T group**), 0.25 % LF + 0.25 % T (**LF-T group**), and 0.5 % L + 0.25 % LF + 0.25 % T (**L-LF-T group**). The experimental period lasted for 120 d, during which all dietary feed additives (CHUP), sourced from Kangxing Pharmaceutical Co. Ltd. (Zhengzhou, China), and rearing conditions adhered to our previously established standards. The supplementation levels of different herbs in the diet were based on findings from our previous trial (Gui et al., 2023). The experimental diets were formulated in accordance with the layer-feeding standard (NY/T 33-2004), and the composition and nutritional levels of the basal diet are shown in Table 1.

Table 1
Ingredients and nutrient level of the basal diet (%, air-dried).

Ingredients	Content	Nutrient[b]	Level
Corn	62.59	Metabolizable energy (kcal/kg)	2691.32
Soybean meal	23.88	Crude protein	14.23
Limestone powder	7.94	Ether extract	9.66
Soybean oil	0.49	Calcium	3.51
DL-Methionine	0.10	Phosphorus	0.34
Premix[a]	5.00	Lysine	0.71
Total	100.00	Methionine	0.37

[a] The premix provided the following per kg of diets: vitamin A, 140,000 IU; vitamin D_3, 50,000 IU; vitamin E, 480 mg; vitamin K, 3.18 g; vitamin B_1, 63 mg; vitamin B_2, 200 mg; vitamin B_6, 140 mg; vitamin B_{12}, 0.7 mg; nicotinic acid, 1000 mg; D-pantothenic acid, 500 mg; folic acid, 50 mg; D-biotin, 5.0 mg; choline chloride, 900 mg; Fe, 2.0 g; Cu, 0.3 g; Mn, 1.8 g; Zn, 2.0 g; I, 70 mg; Se, 9 mg; and phytase, 3000 IU.
[b] Crude protein and ether extract were measured values, and the others were calculated values.

Sample collection

On d 60 and 120 of the trial, blood samples (5 mL) were randomly collected from the wing vein of one bird per replication (eight birds in each group). The heparin-anticoagulated blood samples were then centrifuged at 3500 × *g* and 4 °C for 10 min to collect the plasma, which was stored at −20 °C for subsequent biochemical parameters assays. Additionally, after slaughter, pectoral muscle samples were collected into sterile centrifuge tubes and stored at −80 °C until further metabolite analyses.

Assessment of meat quality

The pH values in pectoral muscle tissue were measured 24 h post-slaughter using a Russell CD700 portable pH meter (Russell pH Limited, München, Germany). The color attributes of the pectoral muscle, including redness (**a***), yellowness (**b***), and lightness (**L***), were assessed at 45 min post-mortem using a CR410 colorimeter (Konica Minolta Sensing, Inc., Tokyo, Japan). Approximately 10 g of pectoral muscle was placed in sealed plastic bottles and stored at 4 °C for 24 h. After removing surface moisture with absorbent paper, the muscle samples were reweighed. Drip loss was calculated as the percentage of weight loss by comparing the final weight to the initial weight. For cooking loss determination, muscle samples (10 g) were boiled in water bath at 80 °C until the internal temperature reached 75 °C, and then cooled to ambient temperature. Cooking loss was calculated as the percentage of weight reduction after cooking. After determining the cooking loss, the samples were trimmed into cuboidal shapes (3 × 1 × 1 cm) along the direction of the myofibrils. These cuboids were used to measure shear force (**N**) using a GR-150 Warner-Bratzler shear machine (GR Manufacturing, Shakopee, MN). Each muscle sample was measured three times, and the mean value was used as the representative shear force for the sample.

Chemical composition analysis of pectoral muscle

Medium- and long-chain fatty acids and amino acids were quantified by liquid chromatography-tandem mass spectrometry (LC-MS/MS) using a Waters Acquity UPLC system (Waters, Milford, MA) coupled to an AB SCIEX QTRAP® 5500 mass spectrometer (AB SCIEX, Framingham, MA).

Determination of medium- and long-chain fatty acids: A precisely weighed aliquot (100.5 ± 0.5 mg) of pectoral muscle tissue was homogenized in a 2-mL polypropylene centrifuge tube with 800 μL of 50 % acetonitrile/water solution. The mixture was vortexed (1 min) and centrifuged (12,000 × *g*, 4 °C, 15 min). Subsequently, 400 μL of the supernatant was derivatized by sequential addition of 200 μL 3-

nitrophenylhydrazine (200 mM) and 200 μL 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide solution, maintaining a 2:1:1 (v/v/v) ratio. The reaction mixture (800 μL) was vortex-mixed (1 min), incubated at 40 °C for 1 h with intermittent agitation (5-min intervals), and centrifuged (12,000 × *g*, 4 °C, 15 min). The resulting supernatant was filtered through a 0.22-μm nylon filter membrane, diluted 10-fold with 50 % acetonitrile/water containing 100 ng/mL deuterated internal standard (IS), and subjected to LC–MS/MS analysis.

Determination of amino acids: Pectoral muscle samples (112.5 ± 0.5 mg) were homogenized in 10 mL centrifuge tubes and transferred to glass hydrolysis ampoules. Acid hydrolysis was performed by adding 8 mL of 6 M HCl, followed by vortexing (1 min), ultrasonication (4 °C, 30 min), and incubation (-60 °C, 10 min). Ampoules were evacuated under vacuum, flame-sealed, and hydrolyzed at 110 °C for 22 h in a forced-air oven. Post-hydrolysis, 1 mL aliquots of hydrolysate were transferred to 10 mL tubes, flash-frozen (−80 °C, 60 min), and lyophilized to complete dryness. Dried residues were reconstituted in 2 mL of 50 % acetonitrile/water containing 100 ng/mL deuterated tryptophan as IS, vortexed (1 min), sonicated (4 °C, 10 min), and centrifuged (12,000 × *g*, 10 min). Filtered supernatants (0.22 μm membrane) were analyzed using LC–MS/MS.

The nutritional values of the pectoral muscle were evaluated using several indicators, including acidic amino acids (**AAA**), atherogenic index (**AI**), aromatic amino acids (**ArAA**), basic amino acids (**BAA**), branched-chain amino acids (**BCAA**), desirable hypocholesterolemic fatty acids (**DHFA**), the sum of essential amino acids (**EAA**), hypercholesterolemic saturated fatty acids (**HSFA**), non-essential amino acids (**NEAA**), sulfur-containing amino acids (**SAA**), and thrombogenic index (**TI**).

For the analysis of differential metabolites, partial least squares discriminant analysis (**PLS-DA**) and Kyoto Encyclopedia of Genes and Genomes (**KEGG**, https://www.kegg.jp/) pathway analyses were conducted using MetaboAnalyst tools (https://www.metaboanalyst.ca/) following the online protocols. PLS-DA VIP > 1 and $P < 0.05$ were employed in order to identify significantly differential metabolites. Using the OmicStudio tools (https://www.omicstudio.cn/tool), a Spearman's correlation analysis was performed to determine the relationship between the meat quality and differential metabolites.

Determination of plasma biochemical parameters

Albumin (**ALB**), alkaline phosphatase (**ALP**), aspartate aminotransferase (**AST**), calcium (**Ca**), glucose (**GLU**), phosphorus (**P**), triglyceride (**TG**), and total protein (**TP**) levels in plasma were measured using commercially available kits (Beijing Leadman Biochemistry Co., Ltd., Beijing, China) and Cobas c311 Roche automatic biochemical analyzer (F. Hoffmann-La Roche Ltd., Basel, Switzerland).

Network pharmacological analysis

The bioactive compounds of L and LF were sourced from the Traditional Chinese Medicine Systems Pharmacology (**TCMSP**) database (Ru et al., 2014). These compounds were further screened based on established criteria (Ping et al., 2023), a drug likeness (**DL**) ≥ 0.18 and oral bioavailability (**OB**) ≥ 30 %. For the bioactive compounds of T, the HERB database (Fang et al., 2021) was utilized, as T was not available in the TCMSP database. Partial information on the bioactive compounds of CHUP is presented in Table 2. Swiss ADME was conducted for the screening of components of T, and the screening principle was the "Rule of Five (Lipinski, 2016)" of Lipinski. Subsequently, the bioactive compounds those met the screening criteria were then submitted to the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) to obtain their respective SMILE names. Targets associated with bioactive compounds were identified using the SwissTargetPrediction database (Daina et al., 2019), with targets having a ``Probability'' >0.1 being selected. After compiling and removing duplicates, functional targets were then queried through the GeneCards (Stelzer et al., 2016) and OMIM (https://www.omim.org/) databases using the keyword "muscle". After duplicates were eliminated, 385 unique related targets were obtained. The intersection of functional targets and herb targets was identified, and Venn diagrams were generated using the VENNY 2.1 tool (https://bioinfogp.cnb.csic.es/tools/venny/index.html) to visually represent these intersections.

Table 2
The partial bioactive compounds information of Chinese herb ultrafine powder (CHUP).

Name	Source	Chinese herb
(+)-Syringaresinol	HERB	T
(20S)-24-ene-3β,20-diol-3-acetate	TCMSP	LF
1-(4,7,7-trimethyl-3-bicyclo[4.1.0]hept-3-enyl) ethanone	HERB	T
3-carene,4-acetyl	HER	T
3-methoxy-4-hydroxybenzaldehyd	HERB	T
Arachidonic acid	TCMSP	L
Arsanin	HERB	T
Artecalin	HERB	T
Benzenecarboxylic acid	HERB	T
Beta-sitosterol	TCMSP	LF
Desacetylmatricarin	HERB	T
Eriodictyol	TCMSP	LF
Esculetin	HERB	T
Ethyl caffeate	HERB	T
Galeopsin	TCMSP	L
Iso-preleoheterin	TCMSP	L
Isorhamnetin	TCMSP	L
Kaempferol	TCMSP	L/LF
Linolenic acid	HERB	T
Lucidumoside D	TCMSP	LF
Luteolin	TCMSP	LF
Methyl caffeate	HERB	T
Mthyl 4-hydroxyphenylacetate	HERB	T
Myristic acid	HERB	T
Olitoriside_qt	TCMSP	LF
Palmitic acid	HERB	T
Phenylacetic acid	HERB	T
p-Hydroxybenzoate	HERB	T
p-Hydroxyphenylpropionic acid	HERB	T
Preleoheterin	TCMSP	L
Quercetin	TCMSP/ HERB	L/LF/T
Scopoletin	HERB	T
Syringaresinol diglucoside_qt	TCMSP	LF
Taraxinic acid	HERB	T
Taraxinic acid β-glucopyranosyl ester	HERB	T
Trans-p-coumaryl alcohol	HERB	T
ZINC04073977	TCMSP	L

The table only lists bioactive compounds for targets with 'Probability' > 0.10.

The intersection targets were inputted into the STRING database (https://cn.string-db.org/) as "*Gallus gallus*" to predict protein-protein interactions (**PPIs**) at a medium confidence level >0.4. Subsequently, the data obtained were imported into Cytoscape 3.9.1 for visualization, and core targets were identified using the Centiscape 2.2 plug-in. The DAVID database (https://david.ncifcrf.gov/) was used to perform GO functional enrichment analysis and KEGG pathway enrichment analysis based on the identified core targets. Subsequently, bubble diagrams of GO and KEGG pathway enrichment analyses were generated using Bioinformatics (https://www.bioinformatics.com.cn/). Following these steps, three distinct target lists were compiled: (1) bioactive compounds derived from herbs, (2) targets associated with these components, and (3) core targets along with their corresponding pathways. Finally, a comprehensive "drug-compound-target-pathway-meat quality" network was constructed using Cytoscape 3.9.1 software.

Molecular docking

The top three core targets related to meat quality within the PPI

network were selected in molecular docking analyses with the top two bioactive compounds. The 3D structures of the selected bioactive compounds were retrieved from the PubChem database, and their SDF format files were converted to MOL2 format using OpenBabel 3.1.1. The 3D protein structures of the core targets were acquired from the RCSB Protein Data Bank (https://www.rcsb.org/) in PDB format, with docking accuracy validated. The PDB IDs for IL6, PPARA, and HIF1A were 1ALU, 6KAX, and 8K73 with resolutions of 1.9 Å, 1.23 Å, and 2.02 Å, respectively. Molecular docking and binding energy calculation were performed using AutodockTools 4.2.6 and Autodock Vina, and the molecular docking results were visualized using PyMol 3.0.2 software.

Statistical analysis

Statistical analyses were performed using one-way analysis of variance (ANOVA) in SPSS software (version 26.0). Duncan's multiple range test was used to compare the differences between the different treatment groups. Data are presented as means with their standard error of the mean (SEM). A P-value <0.05 was considered statistically significant for all comparisons.

Results

Effects of CHUP on meat quality

The impacts of dietary CHUP supplementation on meat quality are summarized in Table 3. On d 60 of the trial, the LF group had a lower pH_{24h} value than the control group, whereas the L-T and LF-T groups exhibited higher pH_{24h} values than the L, LF, and L-LF-T groups, and the L-LF group showed a higher pH_{24h} value than the LF group ($P < 0.05$). Additionally, drip loss was higher ($P < 0.05$) in the L-T group compared with the other treatment groups on d 60 of the trial. On d 120 of the trial, the LF-T and L-LF-T groups had lower ($P < 0.05$) a* value than the control, L, T, and LF groups. Nevertheless, there were no significant changes in L*, b*, shear force, and cooking loss during the trial ($P > 0.05$).

Effects of CHUP on fatty acid composition in pectoral muscles

The PLS-DA analysis was conducted to highlight the distinctions between groups for clearer visualization (Fig. 1). Medium- and long-chain fatty acid levels in the pectoral muscle of laying hens were increased by CHUP supplementation as the length of supplementing time increased. The PLS-DA results showed that there were distinct separations between the L, T, LF, L-LF, L-LF-T, and control groups on d 60 of the trial (Fig. 1A). Furthermore, a pronounced separation was observed between the treated and control groups on d 120 of the trial (Fig. 1B).

Table 4 displays the fatty acid composition in the pectoral muscle of laying hens on d 60 of the trial. The content of C16:1, C16:1t, C18:0, C18:1n-7, C18:1n-9, C18:3n-6, C19:1t (trans-10), and C24:0 was higher ($P < 0.05$) in the L, T, and L-LF groups compared with the control group. In addition, the content of C20:0 in the L, T, LF, L-T, and L-LF-T groups, C23:0 in the L, T, and LF groups, and C24:0 in the LF and L-LF-T groups were higher ($P < 0.05$), when compared with the control group. The L, T, and L-LF groups had higher monounsaturated fatty acids (**MUFA**), while the T, LF, and L-LF groups had a lower n-6/n-3 PUFA ratio compared with the control group ($P < 0.05$). Moreover, the content of C20:1 and C20:1t in the L, T, LF, L-T, and L-LF-T groups, C20:3n-3 and C20:3n-6 in the L and LF-T groups, and C16:1, C20:5n-3, C22:4n-6, and C22:5n-6 in the L and LF-T groups were higher ($P < 0.05$) compared with the control group. The AI was higher ($P < 0.05$) in the L, T, LF, and L-LF groups, as well as the TI in the L, T, LF, L-T, and L-LF-T groups, when compared with the control group.

Table 5 summarizes the effects of dietary CHUP on fatty acid composition in the pectoral muscle of laying hens on d 120 of the trial. The content of C20:2n-6 and C22:3n-3 in all treatment groups (particularly in the L-LF group), saturated fatty acids (SFA; including C6:0, C14:0, C15:0, C16:0, C18:0, C20:0, C22:0, C23:0, and C24:0) in the L, T, LF, L-LF, and LF-T groups, and MUFA (including C15:1, C15:1t, C16:1, C16:1t, C17:1 (cis-10), C17:1t, C18:1n-9t, C19:1t (trans-7), C20:1, C20:1t, and C24:1) in the L group were higher ($P < 0.05$) compared with the control group. Moreover, n-3 PUFA and PUFA content in all treatment groups (except the L-LF-T group) and the n-6 PUFA content in all treatment groups were higher ($P < 0.05$) compared with the control group. The unsaturated fatty acids (**UFA**) content in the L, L-LF, and LF-T

Table 3
Effects of Chinese herb ultrafine powder (CHUP) on meat quality of laying hens during the late laying period.

Items	Dietary groups								SEM	*P*-values
	Control	L	LF	T	L-LF	L-T	LF-T	L-LF-T		
D 60 of the trial										
Meat color										
a*	12.18	10.06	10.39	10.20	9.59	11.00	11.19	11.08	0.218	0.081
b*	15.04	13.64	15.58	13.99	14.93	15.16	13.16	15.26	0.290	0.340
L*	60.75	63.04	62.98	62.41	63.13	62.38	60.30	60.86	0.339	0.171
Meat tenderness										
Cooking loss (%)	34.01	35.77	34.66	32.85	35.01	34.04	34.59	33.14	0.405	0.687
Drip loss (%)	8.25^{b}	9.50^{b}	7.63^{b}	6.25^{b}	10.75^{b}	17.57^{a}	7.50^{b}	8.13^{b}	0.008	0.038
pH_{24h}	5.85^{ab}	5.73^{bc}	5.69^{c}	5.77^{abc}	5.85^{ab}	5.88^{a}	5.89^{a}	5.74^{bc}	0.017	0.008
Shear force (N)	46.59	40.45	42.91	42.60	45.89	46.99	45.23	49.67	1.137	0.582
D 120 of the trial										
Meat color										
a*	9.98^{a}	10.02^{a}	9.56^{a}	9.91^{a}	9.33^{ab}	9.35^{ab}	8.17^{b}	8.14^{b}	0.173	0.011
b*	11.94	10.08	10.87	11.26	11.88	9.70	11.09	10.16	0.239	0.148
L*	56.22	55.67	56.58	56.62	55.37	55.62	57.56	58.42	0.290	0.114
Meat tenderness										
Cooking loss (%)	33.44	32.67	32.68	33.03	32.88	32.09	30.00	34.59	0.451	0.406
Drip loss (%)	6.43	2.33	1.88	2.56	1.64	2.39	5.14	3.91	0.510	0.186
pH_{24h}	5.86	5.94	5.93	5.87	5.86	5.86	5.81	5.87	0.015	0.361
Shear force (N)	32.61	30.61	29.44	27.20	33.39	30.72	27.81	33.73	0.800	0.316

Data are expressed as means with their SEM ($n = 8$). Means within a row without a common superscript letter are significantly different ($P < 0.05$). a*, redness value; b*, yellowness value; L*, lightness value. Control group was received a basal diet; treatment groups were received a basal diet supplemented with 0.5 % *Leonuri herba* (L group), 0.25 % *Ligustri lucidi fructus* (LF group), 0.25 % *Taraxaci herba* (T group), 0.5 % L + 0.25 % LF (L-LF group), 0.5 % L + 0.25 % T (L-T group), 0.25 % LF + 0.25 % T (LF-T group), and 0.5 % L + 0.25 % LF + 0.25 % T (L-LF-T group), respectively.

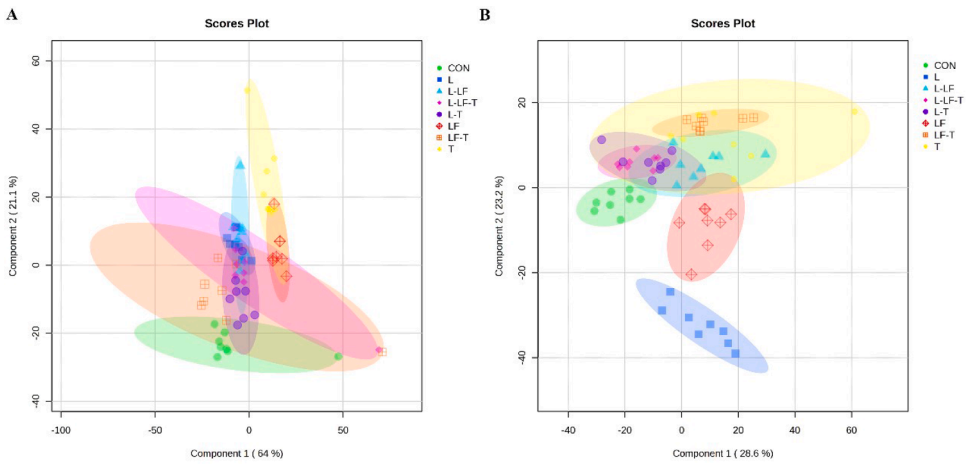

Fig. 1. PLS-DA score plots of fatty acids in the pectoral muscle of laying hens during the late laying period on d 60 (A) and 120 (B) of the trial. Control group was received a basal diet; treatment groups were received a basal diet supplemented with 0.5 % *Leonuri herba* (L group), 0.25 % *Ligustri lucidi fructus* (LF group), 0.25 % *Taraxaci herba* (T group), 0.5 % L + 0.25 % LF (L-LF group), 0.5 % L + 0.25 % T (L-T group), 0.25 % LF + 0.25 % T (LF-T group), and 0.5 % L + 0.25 % LF + 0.25 % T (L-LF-T group), respectively.

groups was higher ($P < 0.05$) compared with the control group. The AI was lower in the L, L-LF, L-T, LF-T, and L-LF-T groups while increasing in the LF group, when compared with the control group ($P < 0.05$). Additionally, the HSFA was decreased in the L-LF-T group while higher in the LF and L-LF groups compared with the control group ($P < 0.05$). Moreover, the DHFA was higher in the L, T, L-LF, and LF-T groups, while the TI was lower in the L, T, LF, L-LF, L-T, and LF-T groups, when compared with the control group ($P < 0.05$).

Effects of CHUP on amino acid composition in pectoral muscles

The PLS-DA analysis results for amino acids are shown in Fig. 2. There was no obvious separation between experimental groups on d 60 (Fig. 2A), and the separation occurred on d 120 of the trial (Fig. 2B).

Amino acid composition in the pectoral muscle of laying hens on d 60 of the trial is presented in Table 6. Compared with the control group, the content of isoleucine, leucine, methionine, phenylalanine, O-phospho-L-serine, ArAA, BCAA, and EAA were higher ($P < 0.05$) in the L, LF, T, L-LF, L-T, and L-LF-T groups. The content of lysine, valine, BAA, and SAA were higher ($P < 0.05$) in the L, T, L-T, and L-LF-T groups compared with the control group. Compared with the control group, the content of histidine, threonine, and NEAA were higher ($P < 0.05$) in the T, L-T, and L-LF-T groups, as well as alanine, arginine, and proline in the L-LF-T group. The content of aspartic acid was higher ($P < 0.05$) in the L, LF, T, L-LF, and L-LF-T groups, as well as glutamic acid in the T group, glycine in the L-T and L-LF-T groups, and AAA in the L, T, and L-LF-T groups, when compared with the control group. Additionally, the content of N-acetyl-L-glutamic acid was lower ($P < 0.05$) in the L, LF, L-LF, and L-LF-T groups, as well as S-adenosyl-L-methionine in the L, LF, T, and L-LF groups, when compared with the control group.

Amino acid composition in the pectoral muscle of laying hens on d 120 of the trial is presented in Table 7. Unlike the changes in amino acid content on d 60 of the trial, dietary L supplementation had a better effect on d 120 of the trial. Dietary L supplementation increased ($P < 0.05$) the content of alanine, arginine, aspartic acid, glutamic acid, glycine, histidine, 1-methyl-L-histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, O-phospho-L-serine, sarcosine, threonine, and valine, when compared with the control group. The content of arginine, glycine, isoleucine, leucine, methionine, phenylalanine, O-phospho-L-serine, AAA, ArAA, BCAA, EAA, and NEAA were higher ($P < 0.05$) in the LF, T, L-LF, L-T, and L-LF-T groups, as well as aspartic acid, lysine, valine, and BAA in the LF, T, L-LF, and L-LF-T groups, when compared with the control group. Moreover, the content of alanine, sarcosine, and SAA were higher ($P < 0.05$) in the LF, L-LF, L-T, and L-LF-T groups, as well as histidine and threonine in the LF and L-LF groups, when compared with the control group. In addition, dietary L-LF supplementation increased ($P < 0.05$) the content of histidine and tryptophan, while LF treatment increased ($P < 0.05$) the content of 1-methyl-L-histidine, when compared with the control group. Additionally, the content of glutamic acid in the L-LF, L-T, and L-LF-T groups and S-adenosyl-L-methionine in the LF and LF-T groups were increased ($P < 0.05$) compared with the control group.

Effects of CHUP on plasma biochemical parameters

Table 8 presents the effects of dietary CHUP on plasma biochemical parameters of laying hens on d 60 and d 120 of the trial. On d 60, the AST level was reduced ($P < 0.05$) in the L, T, LF, and L-LF groups, whereas the GLU level was elevated ($P < 0.05$) in the LF-T and L-LF-T groups, as well as the TG level in the T and L-LF groups; the GLU level was elevated ($P < 0.05$) in the L-T, LF-T, and L-LF-T groups compared with the L, T, LF, and L-LF groups, when compared with the control group. In addition, the TG level was elevated ($P < 0.05$) in the T group compared with the L, L-T, LF-T, and L-LF-T groups, as well as in the L-LF group compared with the L group on d 60 of the trial. On d 120, the ALP level was elevated ($P < 0.05$) in the L-LF group compared with the control, L, LF, LF-T, and L-LF-T groups. The Ca level was elevated ($P < 0.05$) in the LF and LF-T groups compared with the control group, as well as in the LF, LF-T, and L-LF-T groups compared with the T, L-LF, and L-T groups, and in the LF group compared with the L group on d 120 of the trial. However, the P level did not change throughout the trial ($P > 0.05$).

KEGG pathway enrichment and correlation analysis

KEGG pathway enrichment analysis was performed to assess the potential biological roles of 14 key metabolites, as illustrated in Fig. 3. Significant differential metabolites, including tricosanoic acid, palmitelaidic acid, palmitoleic acid, tetracosanoic acid, aspartate, isoleucine, linoleic acid, arachidonic acid, docosanoicacid, stearic acid, glutamate, 3-phospho-serine, arginine, and glycine were enriched by dietary CHUP supplementation. According to KEGG pathway enrichment analysis, the top five differential metabolic pathways in the pectoral muscle of laying hens were arginine biosynthesis, biosynthesis of UFA, histidine metabolism, alanine/aspartate/glutamate metabolism, and glutathione metabolism.

Table 4
Effects of Chinese herb ultrafine powder (CHUP) on fatty acid composition in the pectoral muscle (ng/mg) of laying hens during the late laying period on d 60 of the trial.

Items	Dietary groups								SEM	*P*-values
	Control	L	LF	T	L-LF	L-T	LF-T	L-LF-T		
C6:0	2.86	3.62	3.66	2.67	3.30	3.16	4.98	3.69	0.229	0.303
C7:0	0.47	0.48	0.51	0.63	0.53	0.37	0.98	0.72	0.054	0.113
C8:0	1.12	2.26	2.39	1.86	1.72	1.91	2.70	1.91	0.166	0.420
C11:0	0.11	0.15	0.13	0.17	0.12	0.09	0.10	0.19	0.017	0.852
C12:0	0.13	0.23	0.21	0.32	0.25	0.18	0.13	0.10	0.022	0.170
C14:0	2.52	3.45	2.68	3.38	3.48	2.92	2.72	2.46	0.128	0.208
C14:1t	0.27^{ab}	0.38^{a}	0.33^{ab}	0.32^{ab}	0.38^{a}	0.21^{b}	0.29^{ab}	0.24^{b}	0.015	0.029
C15:0	1.61^{ab}	1.61^{ab}	1.42^{ab}	1.95^{a}	1.40^{ab}	1.11^{b}	1.08^{b}	1.14^{b}	0.075	0.035
C15:1	0.27	0.32	0.25	0.26	0.28	0.24	0.22	0.21	0.015	0.654
C15:1t	0.22	0.29	0.21	0.28	0.24	0.19	0.14	0.11	0.017	0.110
C16:0	30.58	37.39	32.56	39.44	40.34	35.11	35.20	34.56	0.884	0.083
C16:1	10.44^{e}	16.69^{bc}	16.13^{bcd}	20.82^{a}	18.54^{ab}	13.96^{cd}	15.43^{bcd}	13.06^{de}	0.541	<0.001
C16:1t	9.94^{e}	16.48^{bc}	15.86^{bcd}	20.66^{a}	18.18^{ab}	13.42^{cde}	15.22^{bcd}	12.41^{de}	0.560	<0.001
C17:0	1.27^{ab}	1.47^{a}	1.08^{b}	1.48^{a}	1.44^{a}	1.24^{ab}	1.10^{b}	1.28^{ab}	0.039	0.039
C17:1 (cis-10)	1.01	1.13	0.87	1.25	1.11	0.99	0.86	0.93	0.035	0.053
C17:1t	0.89	1.05	0.79	1.15	1.01	0.90	0.76	0.83	0.035	0.052
C18:0	93.57^{c}	130.66^{a}	99.74^{bc}	118.50^{ab}	122.52^{ab}	108.65^{abc}	101.78^{bc}	105.08^{bc}	2.896	0.012
C18:1n-7	0.58^{d}	0.81^{b}	0.52^{d}	0.76^{bc}	1.04^{a}	0.59^{d}	0.65^{cd}	0.63^{d}	0.025	<0.001
C18:1n-9	18.85^{d}	23.52^{abc}	19.30^{cd}	24.48^{ab}	25.21^{a}	22.27^{abcd}	20.27^{bcd}	22.91^{abcd}	0.535	0.009
C18:1n-9t	10.96^{c}	13.39^{abc}	11.14^{c}	13.94^{ab}	14.03^{a}	12.67^{abc}	11.58^{bc}	12.93^{abc}	0.292	0.023
C18:2n-6	64.71	64.73	48.64	64.48	60.44	57.65	69.39	59.27	1.629	0.060
C18:3n-3	1.37^{cd}	1.63^{bc}	1.15^{d}	2.08^{a}	2.12^{a}	1.49^{cd}	1.88^{ab}	1.53^{bc}	0.057	<0.001
C18:3n-6	0.96^{e}	1.33^{cd}	0.91^{e}	2.50^{a}	1.72^{b}	1.10^{de}	1.48^{bc}	1.13^{de}	0.073	<0.001
C19:1t (trans-7)	2.79^{cd}	3.60^{b}	2.26^{d}	4.68^{a}	3.55^{bc}	3.33^{bc}	2.82^{cd}	2.94^{bcd}	0.119	<0.001
C19:1t (trans-10)	3.04^{c}	3.94^{b}	2.95^{c}	4.84^{a}	3.97^{b}	3.66^{bc}	3.02^{c}	3.27^{bc}	0.112	<0.001
C20:0	2.07^{d}	3.19^{a}	2.88^{abc}	3.02^{ab}	2.16^{cd}	3.36^{a}	2.34^{bcd}	3.20^{a}	0.103	0.001
C20:1	2.97^{d}	4.41^{a}	3.93^{abc}	4.23^{a}	3.38^{bcd}	4.19^{a}	3.18^{cd}	4.02^{ab}	0.106	0.001
C20:1t	3.04^{d}	4.45^{a}	4.03^{abc}	4.31^{a}	3.48^{bcd}	4.27^{ab}	3.28^{cd}	4.10^{ab}	0.106	0.001
C20:2n-6	3.32^{abc}	3.79^{a}	2.51^{c}	3.29^{abc}	2.70^{bc}	3.06^{abc}	3.55^{ab}	3.07^{abc}	0.102	0.028
C20:3n-3	3.65^{cd}	5.02^{a}	2.76^{d}	3.90^{bc}	3.80^{c}	4.25^{abc}	4.82^{ab}	3.49^{cd}	0.136	<0.001
C20:3n-6	3.93^{c}	5.29^{a}	2.89^{d}	4.08^{bc}	4.03^{bc}	4.48^{abc}	5.07^{ab}	3.76^{cd}	0.145	<0.001
C20:4n-6	72.85	70.66	55.46	61.63	72.85	73.20	75.60	63.67	1.912	0.085
C20:5n-3	0.68^{d}	0.74^{d}	0.70^{d}	2.43^{a}	0.99^{bc}	1.02^{b}	0.87^{bcd}	0.82^{cd}	0.071	<0.001
C21:0	9.06	7.28	6.14	13.96	15.23	14.92	8.97	8.16	1.362	0.524
C22:0	60.95	61.32	48.56	54.14	61.23	60.48	61.55	52.98	1.530	0.219
C22:1n-9	0.56	0.63	0.54	0.52	0.61	0.69	0.65	0.67	0.031	0.838
C22:1t	0.25	0.31	0.25	0.42	0.37	0.47	0.26	0.42	0.032	0.460
C22:2n-6	0.21	0.24	0.26	0.27	0.23	0.22	0.22	0.24	0.006	0.166
C22:3n-3	13.64	12.87	13.44	15.84	16.05	14.33	14.50	14.94	0.399	0.441
C22:4n-6	2.85^{bcd}	2.41^{d}	2.83^{cd}	3.23^{abc}	3.49^{abc}	3.90^{a}	3.27^{abc}	3.56^{ab}	0.094	0.001
C22:5n-3	41.77^{b}	43.38^{b}	37.16^{b}	44.69^{b}	55.77^{a}	41.44^{b}	45.79^{b}	40.57^{b}	1.311	0.022
C22:5n-6	3.41^{bc}	2.97^{c}	3.45^{bc}	3.64^{abc}	4.03^{ab}	4.42^{a}	3.49^{bc}	4.04^{ab}	0.108	0.020
C23:0	22.27^{cd}	32.04^{a}	29.59^{ab}	31.34^{a}	25.61^{bc}	22.68^{cd}	19.80^{d}	24.14^{cd}	0.778	<0.001
C24:0	2.69^{c}	7.88^{a}	7.27^{ab}	7.53^{ab}	6.00^{ab}	4.34^{bc}	4.51^{abc}	6.41^{ab}	0.414	0.010
C24:1	3.83	4.76	4.30	5.16	4.83	4.77	4.18	4.74	0.132	0.232
ΣSFA	231.26	293.03	238.80	280.40	285.31	260.49	247.93	246.01	5.918	0.051
ΣMUFA	69.91^{c}	96.16^{ab}	83.66^{bc}	108.08^{a}	100.22^{ab}	86.80^{bc}	82.80^{bc}	84.41^{bc}	2.406	0.001
Σn-3 PUFA	61.11	63.65	55.21	68.94	78.73	62.55	67.85	61.35	1.862	0.077
Σn-6 PUFA	152.24	151.41	116.95	143.12	149.50	148.02	162.07	138.75	3.868	0.161
n-6/n-3 PUFA	2.58^{a}	2.38^{ab}	2.12^{bc}	2.08^{bc}	1.90^{c}	2.38^{ab}	2.59^{a}	2.39^{ab}	0.046	<0.001
ΣPUFA	213.36	215.05	172.16	212.06	228.23	210.56	229.92	200.10	5.604	0.240
ΣUFA	283.27	311.22	255.82	320.14	328.44	297.37	312.72	284.51	7.610	0.286
AI	0.14^{b}	0.16^{a}	0.17^{a}	0.17^{a}	0.16^{a}	0.16^{ab}	0.15^{ab}	0.16^{ab}	0.002	0.021
DHFA	376.84	441.87	355.56	438.65	450.97	406.02	414.50	389.59	10.304	0.219
HSFA	33.10	40.83	35.25	42.82	43.82	38.03	37.91	37.02	0.974	0.074
TI	0.43^{c}	0.54^{a}	0.51^{ab}	0.49^{b}	0.46^{bc}	0.48^{b}	0.46^{bc}	0.49^{b}	0.006	<0.001

Data are expressed as means with their SEM ($n = 8$). Means within a row without a common superscript letter are significantly different ($P < 0.05$). ΣSFA, sum of saturated fatty acids; ΣMUFA, sum of monounsaturated fatty acids; Σn-3 PUFA, sum of n-3 polyunsaturated fatty acids; Σn-6 PUFA, sum of n-6 PUFA; n-6/n-3 PUFA, ratio of Σn-6 PUFA to Σn-3 PUFA; ΣPUFA, sum of PUFA; ΣUFA, sum of unsaturated fatty acids; AI, atherogenic index; TI, thrombogenic index; DHFA, desirable hypocholesterolemic fatty acids; HSFA, hypercholesterolemic saturated fatty acids. Control group was received a basal diet; treatment groups were received a basal diet supplemented with 0.5 % *Leonuri herba* (L group), 0.25 % *Ligustri lucidi fructus* (LF group), 0.25 % *Taraxaci herba* (T group), 0.5 % L + 0.25 % LF (L-LF group), 0.5 % L + 0.25 % T (L-T group), 0.25 % LF + 0.25 % T (LF-T group), and 0.5 % L + 0.25 % LF + 0.25 % T (L-LF-T group), respectively.

Spearman's correlation analysis was employed to explore the relationship between meat quality, plasma biochemical parameters, and the content of differential metabolites in the pectoral muscle of laying hens (Fig. 4). Compared with differential amino acids, several differential fatty acids were significantly correlated with meat quality indexes and plasma biochemical parameters. Palmitelaidic acid and palmitoleic acid exhibited a negative correlation ($P < 0.05$) with plasma levels of AST, ALP, GLU, TP, ALB, TG, Ca, and P, while displayed a positive correlation ($P < 0.01$) with a* value. Docosanoic acid showed a negative correlation ($P < 0.01$) with plasma AST level, whereas glutamate showed a positive correlation ($P < 0.05$) with plasma ALP level. In addition, stearic acid was negatively correlated ($P < 0.01$) with pH_{24h}, while it was positively

Table 5
Effects of Chinese herb ultrafine power (CHUP) on fatty acid composition in the pectoral muscle (ng/mg) of laying hens during the late laying period on d 120 of the trial.

Items	Dietary groups								SEM	*P*-values
	Control	L	LF	T	L-LF	L-T	LF-T	L-LF-T		
C6:0	2.85^{a}	1.58^{b}	1.64^{b}	2.22^{ab}	2.09^{ab}	2.90^{a}	2.81^{a}	2.51^{ab}	0.127	0.023
C7:0	0.41	0.41	0.21	0.31	0.35	0.34	0.38	0.36	0.032	0.836
C8:0	1.40	0.60	1.34	1.47	1.04	1.29	1.05	1.29	0.127	0.761
C11:0	0.11	0.09	0.07	0.10	0.07	0.09	0.07	0.08	0.008	0.870
C12:0	0.17	0.17	0.23	0.17	0.25	0.21	0.17	0.17	0.022	0.954
C14:0	3.23^{cde}	4.50^{a}	3.42^{bcd}	3.45^{bcd}	3.98^{ab}	2.90^{de}	3.54^{bc}	2.70^{e}	0.093	<0.001
C14:1t	0.52^{a}	0.48^{ab}	0.26^{c}	0.28^{c}	0.28^{c}	0.23^{c}	0.37^{bc}	0.23^{c}	0.021	<0.001
C15:0	1.34^{d}	2.25^{a}	1.70^{bc}	1.69^{bc}	1.87^{b}	1.38^{cd}	1.92^{b}	1.43^{cd}	0.052	<0.001
C15:1	0.24^{b}	0.40^{a}	0.28^{b}	0.29^{b}	0.38^{a}	0.21^{b}	0.28^{b}	0.21^{b}	0.013	<0.001
C15:1t	0.17^{d}	0.36^{a}	0.27^{bc}	0.27^{bc}	0.34^{ab}	0.17^{d}	0.25^{c}	0.16^{d}	0.013	<0.001
C16:0	41.98^{bc}	44.20^{ab}	46.17^{a}	44.27^{ab}	45.42^{a}	39.12^{cd}	41.10^{bcd}	38.16^{d}	0.491	<0.001
C16:1	22.18^{b}	30.22^{a}	19.30^{c}	17.77^{de}	18.95^{cd}	16.69^{e}	18.59^{cd}	12.78^{f}	0.617	<0.001
C16:1t	22.34^{b}	30.91^{a}	19.06^{c}	17.51^{cd}	18.52^{c}	16.65^{d}	18.42^{c}	12.35^{e}	0.658	<0.001
C17:0	1.26^{e}	1.47^{cd}	1.31^{e}	1.62^{b}	1.79^{a}	1.26^{e}	1.59^{bc}	1.44^{d}	0.026	<0.001
C17:1 (cis-10)	1.00^{c}	1.45^{a}	1.05^{bc}	1.14^{b}	1.48^{a}	0.93^{c}	1.17^{b}	0.98^{c}	0.029	<0.001
C17:1t	0.92^{cd}	1.38^{a}	0.98^{bc}	1.07^{b}	1.39^{a}	0.85^{d}	1.07^{b}	0.88^{cd}	0.028	<0.001
C18:0	127.23^{c}	111.94^{d}	123.35^{c}	142.35^{b}	152.69^{a}	127.62^{c}	131.32^{c}	130.50^{c}	1.736	<0.001
C18:1n-7	0.90^{a}	0.82^{bc}	0.67^{d}	0.85^{abc}	0.88^{ab}	0.70^{d}	0.79^{c}	0.57^{e}	0.016	<0.001
C18:1n-9	26.94^{ab}	28.67^{a}	24.85^{cd}	26.45^{bc}	28.23^{ab}	23.60^{d}	24.58^{d}	23.81^{d}	0.307	<0.001
C18:1n-9t	15.58^{b}	17.68^{a}	15.67^{b}	16.18^{b}	16.04^{b}	13.87^{c}	14.36^{c}	14.18^{c}	0.198	<0.001
C18:2n-6	66.61^{d}	83.60^{b}	73.43^{c}	73.19^{c}	90.69^{a}	69.85^{cd}	81.15^{b}	74.27^{c}	1.109	<0.001
C18:3n-3	1.70^{ab}	1.72^{ab}	1.07^{c}	1.39^{bc}	1.77^{a}	1.69^{ab}	1.66^{ab}	1.13^{c}	0.049	<0.001
C18:3n-6	1.32^{c}	1.76^{a}	1.16^{d}	1.36^{c}	1.49^{b}	1.50^{b}	1.67^{a}	1.02^{e}	0.031	<0.001
C19:1t (trans-7)	3.31^{b}	4.00^{a}	2.56^{d}	3.85^{a}	3.54^{b}	4.01^{a}	4.00^{a}	2.99^{c}	0.073	<0.001
C19:1t (trans-10)	3.87^{c}	4.88^{a}	3.52^{d}	4.41^{b}	4.23^{b}	4.53^{b}	4.49^{b}	3.68^{cd}	0.067	<0.001
C20:0	3.35^{bcd}	4.12^{a}	4.14^{a}	3.29^{cd}	3.59^{bc}	3.19^{cd}	2.99^{d}	3.75^{ab}	0.069	<0.001
C20:1	4.30^{de}	4.94^{a}	4.63^{abc}	4.12^{e}	4.55^{bcd}	4.34^{cde}	4.12^{e}	4.76^{ab}	0.050	<0.001
C20:1t	4.27^{bc}	4.84^{a}	4.57^{ab}	4.11^{c}	4.57^{ab}	4.34^{bc}	4.21^{c}	4.81^{a}	0.049	<0.001
C20:2n-6	3.20^{d}	4.04^{b}	3.48^{c}	3.47^{c}	4.67^{a}	3.88^{b}	4.52^{a}	4.03^{b}	0.069	<0.001
C20:3n-3	4.44^{c}	6.37^{ab}	4.36^{c}	4.35^{c}	6.20^{b}	6.48^{ab}	6.60^{a}	4.18^{c}	0.138	<0.001
C20:3n-6	4.49^{c}	6.45^{b}	4.35^{c}	4.34^{c}	6.30^{b}	6.68^{ab}	6.83^{a}	4.29^{c}	0.146	<0.001
C20:4n-6	73.45^{c}	90.52^{b}	77.12^{c}	84.84^{b}	106.55^{a}	86.03^{b}	109.21^{a}	78.08^{c}	1.746	<0.001
C20:5n-3	0.84^{cde}	0.93^{c}	0.78^{de}	0.85^{cde}	0.86^{cd}	1.05^{b}	1.16^{a}	0.74^{e}	0.021	<0.001
C21:0	9.44	18.10	16.90	8.34	10.45	26.10	12.50	7.84	2.175	0.408
C22:0	71.11^{e}	94.61^{b}	79.56^{c}	79.96^{c}	97.62^{b}	77.41^{cd}	105.15^{a}	72.54^{de}	1.636	<0.001
C22:1n-9	0.57	0.56	0.43	0.40	0.50	0.53	0.55	0.55	0.021	0.322
C22:1t	0.30^{abc}	0.26^{abc}	0.33^{a}	0.18^{bc}	0.37^{a}	0.16^{c}	0.27^{abc}	0.32^{ab}	0.018	0.044
C22:2n-6	0.32	0.34	0.33	0.27	0.37	0.30	0.30	0.31	0.011	0.412
C22:3n-3	13.49^{d}	17.62^{b}	16.85^{b}	15.37^{c}	18.80^{a}	17.17^{b}	17.14^{b}	17.42^{b}	0.232	<0.001
C22:4n-6	2.39^{d}	3.18^{c}	3.93^{b}	2.63^{d}	4.30^{a}	3.75^{b}	3.78^{b}	3.20^{c}	0.086	<0.001
C22:5n-3	43.39^{d}	52.60^{bc}	46.12^{d}	55.46^{b}	50.25^{c}	46.16^{d}	67.81^{a}	39.93^{e}	1.085	<0.001
C22:5n-6	2.93^{c}	3.08^{bc}	4.14^{a}	2.91^{c}	4.66^{a}	4.13^{a}	4.16^{a}	3.55^{b}	0.097	<0.001
C23:0	32.19^{c}	80.63^{a}	75.01^{a}	58.37^{b}	33.60^{c}	33.71^{c}	31.56^{c}	34.40^{c}	2.722	<0.001
C24:0	7.17^{b}	43.12^{a}	45.16^{a}	38.96^{a}	9.53^{b}	8.05^{b}	6.13^{b}	7.44^{b}	2.520	<0.001
C24:1	5.31^{c}	5.99^{a}	5.97^{a}	5.82^{ab}	5.61^{abc}	5.43^{bc}	5.30^{c}	5.55^{abc}	0.058	0.002
ΣSFA	303.23^{e}	407.79^{a}	400.19^{ab}	386.57^{ab}	364.32^{bc}	325.57^{de}	342.28^{cd}	304.61^{e}	6.407	<0.001
ΣMUFA	112.72^{b}	137.84^{a}	104.42^{c}	104.69^{c}	109.88^{bc}	97.25^{d}	102.81^{cd}	88.82^{e}	1.871	<0.001
n-3 ΣPUFA	63.86^{d}	79.26^{b}	69.18^{c}	77.42^{b}	77.88^{b}	72.55^{c}	94.36^{a}	63.40^{d}	1.293	<0.001
n-6 ΣPUFA	154.69^{d}	192.99^{b}	167.94^{c}	172.99^{c}	219.02^{a}	176.13^{c}	211.62^{a}	168.75^{c}	3.044	<0.001
n-6/n-3 PUFA	2.42	2.43	2.43	2.23	2.81	2.43	2.24	2.66	0.024	0.376
ΣPUFA	218.55^{e}	272.24^{b}	237.12^{cd}	250.42^{c}	296.91^{a}	248.67^{cd}	305.98^{a}	232.15^{de}	4.167	<0.001
ΣUFA	331.27^{bc}	410.08^{a}	341.54^{bc}	355.11^{b}	406.78^{a}	345.92^{b}	408.80^{a}	320.97^{c}	5.166	<0.001
AI	0.17^{b}	0.15^{c}	0.18^{a}	0.16^{b}	0.15^{c}	0.15^{c}	0.14^{d}	0.15^{c}	0.002	<0.001
DHFA	458.50^{e}	522.02^{bc}	464.89^{e}	497.46^{cd}	559.48^{a}	473.54^{de}	540.12^{ab}	451.47^{e}	5.991	<0.001
HSFA	45.22^{bcd}	48.70^{ab}	49.59^{a}	47.72^{abc}	49.40^{a}	42.02^{de}	44.64^{cd}	40.87^{e}	0.558	<0.001
TI	0.53^{a}	0.40^{d}	0.50^{b}	0.51^{b}	0.51^{b}	0.48^{c}	0.40^{d}	0.54^{a}	0.007	<0.001

Data are expressed as means with their SEM ($n = 8$). Means within a row without a common superscript letter are significantly different ($P < 0.05$). ΣSFA, sum of saturated fatty acids; ΣMUFA, sum of monounsaturated fatty acids; Σn-3 PUFA, sum of n-3 polyunsaturated fatty acids; Σn-6 PUFA, sum of n-6 PUFA; n-6/n-3 PUFA, ratio of Σn-6 PUFA to Σn-3 PUFA; ΣPUFA, sum of PUFA; ΣUFA, sum of unsaturated fatty acids; AI, atherogenic index; TI, thrombogenic index; DHFA, desirable hypocholesterolemic fatty acids; HSFA, hypercholesterolemic saturated fatty acids. Control group was received a basal diet; treatment groups were received a basal diet supplemented with 0.5 % *Leonuri herba* (L group), 0.25 % *Ligustri lucidi fructus* (LF group), 0.25 % *Taraxaci herba* (T group), 0.5 % L + 0.25 % LF (L-LF group), 0.5 % L + 0.25 % T (L-T group), 0.25 % LF + 0.25 % T (LF-T group), and 0.5 % L + 0.25 % LF + 0.25 % T (L-LF-T group), respectively.

correlated ($P < 0.05$) with plasma AST and ALP levels and muscle b* value. Moreover, tricosanoic acid and glycine were positively correlated ($P < 0.05$) with a* value and plasma AST level, respectively; while those were negatively correlated ($P < 0.05$) with plasma GLU level and shear force, respectively.

Exploring the potential mechanism of CHUP promoting nutritional value of meat based on network pharmacology analysis

The TCMSP database was used to identify the bioactive compounds associated with L and LF, while the HERB database was used to identify the drug constituents of T. A total of six bioactive compounds were identified for L, seven for LF, and 23 for T. Furthermore, 343 targets

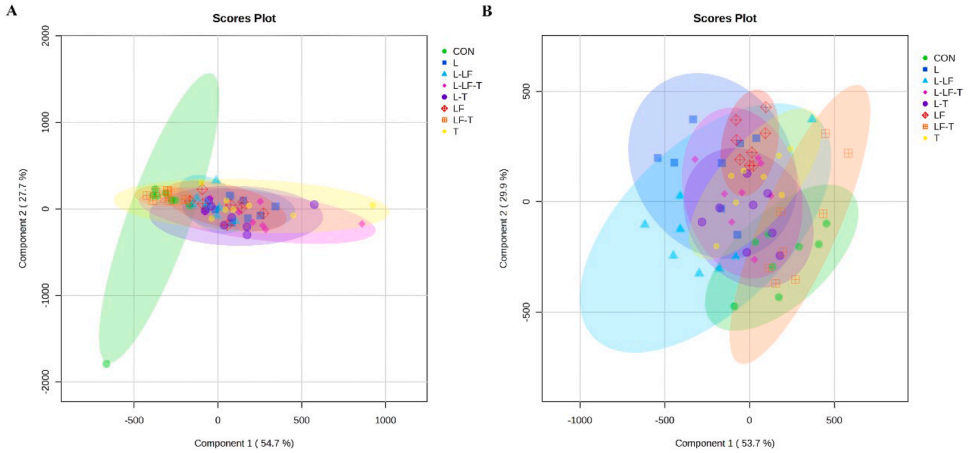

Fig. 2. PLS-DA score plots of amino acids in the pectoral muscle of laying hens during the late laying period on d 60 (A) and 120 (B) of the trial. Control group was received a basal diet; treatment groups were received a basal diet supplemented with 0.5 % *Leonuri herba* (L group), 0.25 % *Ligustri lucidi fructus* (LF group), 0.25 % *Taraxaci herba* (T group), 0.5 % L + 0.25 % LF (L-LF group), 0.5 % L + 0.25 % T (L-T group), 0.25 % LF + 0.25 % T (LF-T group), and 0.5 % L + 0.25 % LF + 0.25 % T (L-LF-T group), respectively.

were obtained for L, 164 targets for LF, and 379 targets for T. After the removing duplicate values, 483 unique targets for these herbs were identified. The bioactive compounds and targets of the three herbs were imported into Cytoscape to construct a ``Herb-compound-target'' network (Fig. 5A).

The NCBI database was searched using the keyword "muscle" with the species specified as "*Gallus gallus*". After eliminating duplicates, 382 targets related to meat quality were identified. The intersection of the targets associated with the compounds of L-LF-T and the targets related to meat quality was determined, resulting in 21 overlapping targets (Fig. 5B). To identify core targets, these 21 overlapping targets were input into the STRING database (Fig. 5C). Targets with interaction scores lower than 0.40 were removed, and the remaining nine targets were considered as the potential key targets. These nine targets were further analyzed in Cytoscape to construct a PPI network, and topology analysis was performed using Centiscape (Fig. 5D).

The nine core targets were then subjected to functional enrichment analysis using the DAVID database (Fig. 5E). The analysis revealed that the bioactive compounds of L-LF-T affecting meat quality were associated with 10 key pathways, including long-chain fatty acid binding, PPAR signaling pathway, fatty acid transport, fatty acid binding, nucleus, animal organ development, response to hypoxia, heparin-binding, extracellular region, and zinc ion binding.

Moreover, an interactive "drug-compound-target-pathway-meat quality" network was constructed (Fig. 5F). Following the network topology analysis, the top two relevant bioactive compounds and their corresponding targets through which the herbs exert their roles were filtered according to the size ranking of the "degree" values. The results indicated that the regulation of meat quality by L-LF-T may be associated with linolenic acid and arachidonic acid, as well as key targets such as peroxisome proliferator-activated receptor alpha (PPARA), interleukin 6 (IL6), and hypoxia-inducible factor 1 subunit alpha (HIF1A).

Molecular docking of bioactive compound and key cores

Molecular docking simulations were conducted to investigate the binding interactions of linolenic acid and arachidonic acid with the proteins PPARA, IL6, and HIF1A. The lowest binding energies calculated for the interaction of the PPARA protein with linolenic acid and arachidonic acid were −3.23 and −0.41 kcal/mol, respectively (Fig. 6A, D). Similarly, for the IL6 protein, the lowest binding energies with linolenic acid and arachidonic acid were −1.53 and 0.39 kcal/mol, respectively (Fig. 6B, E). In addition, for the HIF1A protein, the lowest binding energies for linolenic acid and arachidonic acid were −2.25 and 0.24 kcal/mol, respectively (Fig. 6C, F). Furthermore, linolenic acid was found to form hydrogen bonds with the residues PRO-417 on the PPARA protein, ARG-179 and ARG-182 on the IL6 protein, and ASP-201 and ARG-238 on the HIF1A protein. Moreover, arachidonic acid established hydrogen bonds with the residues GLU-212 and LYS-216 on the PPARA protein, LYS-70 on the IL6 protein, and ASN-171 on the HIF1A protein. These interactions highlight the distinct binding mechanism of the two fatty acids with the target proteins.

Discussion

Laying hens experience physiological changes that often lead to a decline in meat quality, characterized by tougher texture and inferior taste during the late laying period. In light of the comprehensive ban on antibiotics, Chinese herbs emerge as a green and safe alternative, offering significant practical benefits and broad application potential for enhancing the meat quality of aged laying hens. Consequently, this research delves into the mechanism by which dietary CHUP supplementation affects meat quality and nutritional values in aged laying hens, leveraging network pharmacology analysis and molecular docking. The findings indicate that dietary CHUP supplementation increased the UFA content in the pectoral muscle of laying hens, potentially through to the PPAR signaling pathway regulated by linolenic acid and arachidonic acid.

Chicken meat with low-fat content is widely regarded by consumers as a high-quality protein source (Sun et al., 2024). The meat quality attributes, such as water holding capacity (**WHC**), pH, and color, significantly influence consumer preferences (Kamruzzaman et al., 2012). pH plays a crucial role in determining fresh meat quality characteristics, including shelf life, juiciness, tenderness, and color (Liu et al., 2021). The WHC is a key functional characteristic of fresh meat and is directly linked to meat tenderness (Mir et al., 2017). Drip loss, a major component of WHC, leads to the loss of specific vitamins, amino acids, and certain sensory quality attributes of meat (Sun et al., 2024). In the present study, dietary LF supplementation reduced the pH_{24h} value, while L-T supplementation increased drip loss on d 60 of the trial, indicating a reduction in WHC in the pectoral muscle of laying hens. However, these feed additives had no significant effect on pH and drip loss on d 120 of the trial. These results indicate that long-term dietary CHUP supplementation does not significantly affect pH_{24h} and drip loss in the pectoral muscle of laying hens. Similarly, Wang et al. (2023) also reported that dietary T supplementation had no significant impact on pH_{24h} and drip loss in the *pectoralis major* muscle of Arbour Acres broiler chickens. Additionally, dietary LF-T and L-LF-T supplementation

Table 6
Effects of Chinese herb ultrafine power (CHUP) on amino acid composition in the pectoral muscle (ng/mg) of laying hens during the late laying period on d 60 of the trial.

Items	Dietary groups								SEM	*P*-values
	Control	L	LF	T	L-LF	L-T	LF-T	L-LF-T		
Ac-Glu-OH	2.13^{a}	1.89^{b}	1.85^{b}	2.13^{a}	1.85^{b}	1.97^{ab}	2.00^{ab}	1.85^{b}	0.028	0.019
Ac-Lys-OH	0.55	0.42	0.35	0.46	0.32	0.49	0.50	0.61	0.028	0.134
Ala	607.72^{bc}	731.38^{ab}	701.05^{ab}	714.25^{ab}	628.60^{abc}	719.35^{ab}	576.17^{c}	733.97^{a}	14.836	0.021
Arg	403.00^{bc}	539.29^{ab}	484.80^{ab}	554.48^{ab}	501.30^{ab}	545.67^{ab}	279.56^{c}	612.89^{a}	22.735	0.005
Asp	44.40^{d}	90.30^{ab}	88.82^{ab}	116.03^{a}	80.51^{bc}	71.41^{bcd}	56.85^{cd}	95.97^{ab}	4.250	<0.001
Cys	73.36	78.75	80.23	84.53	73.84	84.67	76.04	85.59	2.225	0.760
DMG	0.84	0.95	1.25	1.15	0.43	1.36	1.37	1.44	0.143	0.680
Glu	264.69^{bc}	353.11^{ab}	306.20^{abc}	391.65^{a}	301.92^{abc}	343.47^{ab}	227.28^{c}	373.72^{ab}	13.364	0.023
Gly	509.10^{bc}	629.49^{ab}	616.56^{ab}	701.97^{ab}	584.16^{ab}	751.63^{a}	372.71^{c}	765.74^{a}	26.393	0.001
His	204.69^{c}	270.98^{abc}	247.73^{bc}	275.72^{ab}	257.54^{bc}	283.23^{ab}	218.37^{bc}	324.71^{a}	8.371	0.007
His(1-met)-OH	10.12	13.39	12.04	10.61	9.88	11.31	8.56	12.78	0.671	0.675
HYP	5.94	2.33	2.96	3.07	2.58	2.70	2.68	2.77	0.433	0.529
Ile	125.47^{c}	248.92^{ab}	217.62^{b}	289.33^{a}	204.19^{b}	231.42^{ab}	108.38^{c}	255.99^{ab}	10.234	<0.001
Leu	134.02^{c}	246.31^{a}	222.62^{ab}	255.10^{a}	190.99^{b}	233.37^{ab}	113.63^{c}	252.79^{a}	8.476	<0.001
Lys	347.16^{cd}	493.79^{ab}	432.37^{bc}	525.01^{ab}	410.50^{bc}	492.87^{ab}	243.83^{d}	557.57^{a}	17.819	<0.001
Met	24.22^{c}	46.48^{ab}	43.24^{b}	48.28^{ab}	40.51^{b}	47.26^{ab}	24.63^{c}	53.85^{a}	1.599	<0.001
Orn	188.37	107.82	106.29	55.40	68.38	93.18	86.89	92.97	13.443	0.365
pGlu	4.41	4.67	4.38	4.83	4.27	4.71	2.99	4.33	0.236	0.659
Phe	144.97^{c}	261.98^{ab}	222.76^{ab}	266.63^{ab}	213.10^{b}	248.43^{ab}	135.59^{c}	271.94^{a}	8.666	<0.001
Pro	137.40^{b}	172.02^{ab}	167.12^{ab}	184.04^{ab}	156.85^{b}	177.89^{ab}	138.74^{b}	212.62^{a}	5.824	0.019
pSer	632.26^{c}	1093.94^{ab}	993.75^{b}	1128.70^{ab}	893.76^{b}	1096.34^{ab}	597.55^{c}	1245.41^{a}	37.412	<0.001
SAH	0.90	1.55	1.09	1.17	0.80	1.90	0.78	1.57	0.129	0.263
SAM	362.91^{a}	199.67^{b}	167.05^{b}	203.16^{b}	176.63^{b}	353.00^{a}	366.34^{a}	289.55^{ab}	18.473	0.003
Sar	625.81^{ab}	744.57^{a}	723.05^{a}	729.56^{a}	638.84^{ab}	735.88^{a}	584.85^{b}	730.89^{a}	14.707	0.019
Ser	1.00	1.02	0.34	0.76	0.51	0.49	0.35	1.00	0.095	0.289
Thr	47.56^{c}	59.88^{bc}	57.53^{bc}	71.90^{ab}	55.48^{bc}	70.07^{ab}	43.53^{c}	82.05^{a}	2.778	0.004
Trp	49.67	54.09	62.67	62.28	55.27	61.51	63.06	69.25	1.912	0.227
Tyr	3.78	4.66	6.15	4.77	4.61	5.90	6.02	4.83	0.449	0.892
Val	40.10^{de}	68.79^{abc}	61.35^{bc}	81.19^{a}	57.21^{cd}	66.16^{abc}	31.66^{e}	78.96^{ab}	2.900	<0.001
AAA	309.10^{b}	443.41^{a}	395.03^{ab}	507.69^{a}	382.43^{ab}	414.88^{ab}	284.14^{b}	469.68^{a}	16.607	0.005
ArAA	198.42^{c}	320.74^{ab}	291.59^{ab}	333.68^{ab}	272.98^{b}	315.84^{ab}	204.67^{c}	346.03^{a}	9.830	<0.001
BAA	954.84^{bc}	1304.06^{a}	1164.90^{ab}	1355.21^{a}	1169.34^{ab}	1321.77^{a}	741.77^{c}	1495.17^{a}	44.667	<0.001
BCAA	299.60^{c}	564.01^{ab}	501.59^{ab}	625.61^{a}	452.39^{b}	530.96^{ab}	253.66^{c}	587.74^{a}	21.125	<0.001
EAA	1117.85^{d}	1751.21^{abc}	1567.89^{bc}	1875.43^{ab}	1484.79^{c}	1734.33^{abc}	982.67^{d}	1947.13^{a}	56.397	<0.001
NEAA	2044.46^{bc}	2600.04^{ab}	2451.28^{ab}	2752.48^{a}	2332.31^{ab}	2700.47^{a}	1733.75^{c}	2886.32^{a}	77.323	0.001
SAA	97.58^{c}	125.23^{ab}	123.47^{abc}	132.81^{a}	114.36^{abc}	131.92^{a}	100.67^{bc}	139.44^{a}	3.337	0.006

Data are expressed as means with their SEM ($n = 8$). Means within a row without a common superscript letter are significantly different ($P < 0.05$). Ac-Glu-OH, N-acetyl-L-glutamic acid; Ac-Lys-OH, N-acetyl-L-lysine; Ala, alanine; Arg, arginine; Asp, aspartic acid; Cys, cystine; DMG, dimethylglycine; Glu, glutamic acid; Gly, glycine; His, histidine; His (1-me)-OH, 1-methyl-L-histidine; HYP, hydroxyproline; Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; Orn, ornithine; pGlu, pyroglutamic acid; Phe, phenylalanine; Pro, proline; pSer, O-phospho-L-serine; SAH, S-adenosyl-L-homocysteine; SAM, S-adenosyl-L-methionine; Sar, sarcosine; Ser, serine; Thr, threonine; Trp, tryptophan; Tyr, tyrosine; Val, valine; AAA, acidic amino acids (aspartic acid and glutamic acid); ArAA, aromatic amino acids (phenylalanine and tyrosine); BAA, basic amino acids (histidine, lysine, and arginine); BCAA, branched-chain amino acids (leucine, isoleucine, and valine); EAA, essential amino acids; NEAA, non-essential amino acids; SAA, sulphur-containing amino acids (methionine and cystine). Control group was received a basal diet; treatment groups were received a basal diet supplemented with 0.5 % *Leonuri herba* (L group), 0.25 % *Ligustri lucidi fructus* (LF group), 0.25 % *Taraxaci herba* (T group), 0.5 % L + 0.25 % LF (L-LF group), 0.5 % L + 0.25 % T (L-T group), 0.25 % LF + 0.25 % T (LF-T group), and 0.5 % L + 0.25 % LF + 0.25 % T (L-LF-T group), respectively.

reduced the a* value on d 120 of the trial, potentially due to the synergistic effects of the bioactive compounds in L, LF, and T, as individual Chinese herbs did not show significant changes the a* value. The underlying mechanism of this formula remains poorly understood and requires further additional research.

In general, dietary fats comprise fatty acids that can exert either favorable or unfavorable effects on the prevention and management of metabolic disorders (Chen and Liu, 2020). Excessive intake of SFA and carbohydrates has been linked to metabolic disorders and cardiovascular diseases (Paslawski et al., 2024). Notably, dietary supplementation with single Chinese herbs, L-LF, and LF-T resulted in higher concentrations of SFAs in the pectoral muscle of laying hens during the long-term supplementation (d 120 of the trial), although no significant effects were observed during the short-term (d 60 of the trial). Numerous studies have indicated that the fatty acid composition of meat can be altered by herbal additives (Valenzuela-Grijalva et al., 2017). For instance, incorporating curcumin and microencapsulated phytogenics into broiler diets has been found to improve meat quality and extend shelf life by increasing the PUFA content (Galli et al., 2020). PUFAs are regarded as one of the most crucial elements of cellular structures, significantly influencing the normal growth and functioning of various organisms (Czumaj and Śledziński, 2020). In the present study, dietary CHUP supplementation notably elevated the PUFA levels (including arachidonic acid and docosapentaenoic acid) on d 120 of the trial, except in the L-LF-T group. Arachidonic acid metabolism was identified as a differentially enriched pathway. Given its role in regulating vascular tone and cardiovascular health, elevated arachidonic acid in the pectoral muscle may promote the production of anti-inflammatory lipid mediators in consumers, aligning with dietary recommendations for cardiovascular health (Zhou et al., 2021). These results suggest that dietary CHUP supplementation may enhance the nutritional value in the pectoral muscle of laying hens.

Human health organizations often recommend substituting of red meat with foods rich in UFA, such as poultry and fish (Hooper et al., 2015). In the present study, long-term supplementation with L, L-LF, and LF-T increased UFA concentrations in the pectoral muscle of laying hens. Furthermore, KEGG pathway enrichment analysis revealed that the differential metabolites involved in UFA biosynthesis. Consuming foods with a favorable fatty acid profile, characterized by lower AI and TI, is associated with better nutritional quality and incidence reduced risk of

Table 7
Effects of Chinese herb ultrafine power (CHUP) on amino acid composition in the pectoral muscle (ng/mg) of laying hens during the late laying period on d 120 of the trial.

Items	Dietary groups								SEM	*P*-values
	Control	L	LF	T	L-LF	L-T	LF-T	L-LF-T		
AC-Glu-OH	2.14^{bc}	2.17^{bc}	2.12b^{c}	2.06^{c}	2.54^{a}	2.09^{bc}	2.41^{ab}	2.01^{c}	0.040	0.006
AC-Lys-OH	0.68	0.79	0.76	0.60	0.64	0.65	0.65	0.57	0.027	0.442
Ala	617.03^{d}	833.52^{a}	779.25^{ab}	691.11^{cd}	775.30^{ab}	715.64^{bc}	618.90^{d}	718.27^{bc}	12.722	<0.001
Arg	395.40^{d}	753.77^{a}	655.21^{b}	595.14^{bc}	617.96^{b}	516.67^{c}	405.95^{d}	607.29^{bc}	17.989	<0.001
Asp	50.82^{de}	109.14^{a}	83.03^{bc}	91.99^{ab}	85.90^{bc}	67.13^{cd}	45.47^{e}	89.94^{ab}	3.494	<0.001
Cys	67.14	79.66	82.69	77.38	72.62	69.70	78.92	77.25	1.563	0.173
DMG	3.65	4.34	3.31	2.99	3.29	3.93	1.90	3.25	0.227	0.267
Glu	233.43^{c}	329.52^{ab}	309.44^{abc}	314.77^{abc}	366.25^{a}	350.02^{a}	258.10^{bc}	349.55^{a}	10.611	0.011
Gly	430.29^{c}	857.46^{a}	857.75^{a}	754.40^{ab}	705.28^{b}	658.93^{b}	428.99^{c}	760.10^{ab}	23.899	<0.001
His	227.47^{c}	362.60^{a}	316.12^{b}	277.50^{bc}	294.79^{b}	231.65^{c}	228.89^{c}	272.73^{bc}	7.848	<0.001
His(1-met)-OH	12.26^{c}	20.76^{a}	18.09^{ab}	14.23^{bc}	13.25^{c}	11.87^{c}	11.07^{c}	12.81^{c}	0.608	<0.001
HYP	2.79	3.68	3.17	3.37	3.60	3.33	2.89	3.09	0.115	0.494
Ile	151.55^{d}	289.49^{a}	267.59^{ab}	235.45^{bc}	286.01^{a}	220.77^{c}	152.85^{d}	237.82^{bc}	7.829	<0.001
Leu	151.61^{c}	286.46^{a}	253.21^{ab}	229.66^{b}	280.53^{a}	233.16^{b}	144.12^{c}	244.77^{ab}	7.879	<0.001
Lys	399.62^{cd}	660.41^{a}	594.71^{ab}	510.86^{b}	634.11^{a}	494.60^{bc}	362.07^{d}	524.56^{b}	17.271	<0.001
Met	31.26^{c}	55.81^{a}	46.33^{b}	44.84^{b}	55.80^{a}	44.18^{b}	30.82^{c}	47.21^{b}	1.412	<0.001
Orn	75.87	86.21	83.94	90.75	66.54	60.08	65.80	94.83	4.394	0.390
pGlu	3.13	5.12	3.79	4.46	6.13	4.60	4.24	4.73	0.293	0.330
Phe	182.08^{c}	288.68^{a}	249.48^{b}	241.91^{b}	285.02^{a}	225.36^{b}	173.76^{c}	244.10^{b}	6.223	<0.001
Pro	217.46^{b}	273.41^{a}	226.15^{ab}	203.36^{b}	247.02^{ab}	232.67^{ab}	202.98^{b}	246.98^{ab}	5.769	0.023
pSer	746.36^{d}	1231.50^{a}	1076.37^{abc}	976.97^{c}	1174.54^{ab}	960.72^{c}	743.04^{d}	1031.11^{bc}	28.673	<0.001
SAH	1.19	1.84	1.62	1.74	1.33	1.68	1.59	1.36	0.070	0.228
SAM	260.12^{cd}	409.56^{abc}	491.72^{a}	308.35^{bcd}	208.02^{d}	251.45^{cd}	421.58^{ab}	277.85^{bcd}	20.619	0.002
Sar	627.17^{d}	839.73^{a}	776.58^{abc}	706.24^{cd}	798.64^{ab}	739.97^{bc}	631.44^{d}	735.88^{bc}	12.718	<0.001
Ser	0.30	1.58	0.56	1.08	1.20	0.61	0.54	0.71	0.125	0.177
Thr	62.34^{bc}	86.66^{a}	85.46^{a}	62.10^{bc}	90.47^{a}	70.64^{abc}	51.46^{c}	74.23^{ab}	2.730	0.001
Trp	83.63^{bc}	98.38^{ab}	103.15^{ab}	68.84^{c}	112.42^{a}	95.62^{ab}	65.09^{c}	80.60^{bc}	3.333	0.001
Tyr	5.97	5.44	3.70	3.60	7.49	3.68	4.04	7.05	0.553	0.416
Val	51.61^{cd}	88.87^{a}	79.80^{ab}	72.38^{ab}	87.33^{a}	66.28^{bc}	46.22^{d}	72.47^{ab}	2.540	<0.001
AAA	284.25^{e}	438.66^{a}	392.47^{ab}	406.76^{cd}	452.14^{abc}	417.15^{cd}	303.57^{de}	439.49^{bcd}	12.663	0.001
ArAA	271.68^{e}	392.50^{a}	356.34^{abc}	314.35^{cd}	404.93^{ab}	324.66^{d}	242.89^{e}	331.75^{bcd}	8.420	<0.001
BAA	1022.49^{d}	1776.79^{a}	1566.04^{b}	1383.50^{bc}	1546.87^{b}	1242.93^{cd}	996.90^{d}	1404.57^{bc}	39.559	<0.001
BCAA	354.77^{d}	664.81^{a}	600.60^{b}	537.49^{bc}	653.88^{b}	520.21^{c}	343.19^{d}	555.06^{bc}	17.814	<0.001
EAA	1341.17^{e}	2217.36^{a}	1995.86^{ab}	1743.54^{cd}	2126.48^{abc}	1682.28^{bc}	1255.27^{de}	1798.48^{bc}	50.202	<0.001
NEAA	2017.85^{b}	3243.51^{a}	2997.78^{a}	2732.84^{a}	2879.03^{a}	2615.05^{a}	2043.89^{b}	2857.13^{a}	64.070	<0.001
SAA	98.40^{d}	135.47^{a}	129.01^{abc}	122.22^{cd}	128.42^{ab}	113.88^{bc}	109.75^{d}	124.45^{bc}	2.263	<0.001

Data are expressed as means with their SEM ($n = 8$). Means within a row without a common superscript letter are significantly different ($P < 0.05$). Ac-Glu-OH, N-acetyl-L-glutamic acid; Ac-Lys-OH, N-acetyl-L-lysine; Ala, alanine; Arg, arginine; Asp, aspartic acid; Cys, cystine; DMG, dimethylglycine; Glu, glutamic acid; Gly, glycine; His, histidine; His (1-me)-OH, 1-methyl-L-histidine; HYP, hydroxyproline; Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; Orn, ornithine; pGlu, pyroglutamic acid; Phe, phenylalanine; Pro, proline; pSer, O-phospho-L-serine; SAH, S-adenosyl-L-homocysteine; SAM, S-adenosyl-L-methionine; Sar, sarcosine; Ser, serine; Thr, threonine; Trp, tryptophan; Tyr, tyrosine; Val, valine; AAA, acidic amino acids (aspartic acid and glutamic acid); ArAA, aromatic amino acids (phenylalanine and tyrosine); BAA, basic amino acids (histidine, lysine, and arginine); BCAA, branched-chain amino acids (leucine, isoleucine, and valine); EAA, essential amino acids; NEAA, non-essential amino acids; SAA, sulphur-containing amino acids (methionine and cystine). Control group was received a basal diet; treatment groups were received a basal diet supplemented with 0.5 % *Leonuri herba* (L group), 0.25 % *Ligustri lucidi fructus* (LF group), 0.25 % *Taraxaci herba* (T group), 0.5 % L + 0.25 % LF (L-LF group), 0.5 % L + 0.25 % T (L-T group), 0.25 % LF + 0.25 % T (LF-T group), and 0.5 % L + 0.25 % LF + 0.25 % T (L-LF-T group), respectively.

coronary heart disease (Chen and Liu, 2020). The present study demonstrated that dietary long-term supplementation with L, L-LF, L-T, and LF-T led to a decrease in both AI and TI in the pectoral muscle of laying hens, particularly with L and LF-T supplementation.

Stachydrine, the main alkaloid component of L, has demonstrated significant biological activity relevant to cardiovascular diseases (Liao et al., 2023). Similarly, specnuezhenide, the primary bioactive compound in LF, has been reported to mitigate age-related hepatic lipid accumulation by enhancing bile acid profiles (Deng et al., 2023). Additionally, dietary T supplementation was found to increase the proportions of C20:2, C20:4n-6, and C22:6n-3, as well as the sum of n-3 PUFA in the *pectoralis major* muscle of broiler chickens (Wang et al., 2023). The findings of the present study suggest that the observed increase in UFA levels may be associated with the bioactive components of Chinese herbs. Thus, consumers can benefit their health by selectively choosing chicken meat to improve UFA intake and protect against metabolic diseases.

Amino acids are essential for protein and peptide synthesis and serve as bioactive compounds that play key roles in signaling cascades and metabolic regulation (Hu and Guo, 2021). In the present study, dietary L-LF-T supplementation showed a greater effect on amino acid concentrations on d 60 of the trial, whereas dietary L supplementation demonstrated a greater benefit of amino acid content on d 120 of the trial. Proteins and amino acids are essential for maintaining bone health, glucose homeostasis, and gastrointestinal functions. Specifically, arginine and lysine have been shown to stimulate bone-forming cell activity and enhance collagen production (Aggarwal and Bains, 2022). The present study demonstrated that dietary supplementation with L, LF, T, L-LF, and L-LF-T increased the content of arginine and lysine on d 120 of the trial, with differential metabolites involved in arginine biosynthesis. A previous study reported that increasing the digestible arginine-to-lysine ratio in feed can positively influence feed efficiency in Ross 308 broilers (Zampiga et al., 2018). Overall, the findings of the current study suggest that dietary CHUP supplementation was found to enhance the nutritional value of amino acids in the pectoral muscle, which might be derived from the feed composition of different CHUP additives.

Plasma biochemical parameters represent the metabolic state of the body in laying hens. The ALP level in the blood reflects endoskeletal growth and development, as well as calcium and phosphorus deposition

Table 8
Effects of Chinese herb ultrafine powder (CHUP) on plasma biochemical parameters of laying hens during the late laying period.

Items	Dietary groups								SEM	*P*-values
	Control	L	LF	T	L-LF	L-T	LF-T	L-LF-T		
D 60 of the trial										
ALB (g/L)	23.54	22.34	24.43	23.86	21.94	23.33	24.33	25.44	0.342	0.206
ALP (U/L)	185.25	184.50	188.43	224.75	168.63	233.86	291.63	156.14	16.567	0.528
AST (U/L)	203.88^{a}	155.25^{b}	160.14^{b}	152.25^{b}	161.63^{b}	177.29^{ab}	185.63^{ab}	188.14^{ab}	4.468	0.021
Ca (mmol/L)	4.90	4.74	5.31	5.47	5.62	5.68	5.27	6.27	0.123	0.061
GLU (mmol/L)	7.25^{bc}	5.54^{c}	6.27^{c}	6.48^{c}	5.55^{c}	8.71^{ab}	10.45^{a}	9.14^{a}	0.298	<0.001
P (mmol/L)	2.24	2.31	2.81	2.37	2.35	2.55	2.21	2.50	0.062	0.296
TG (mmol/L)	11.26^{c}	10.35^{c}	15.66^{abc}	20.78^{a}	17.55^{ab}	12.56^{bc}	13.28^{bc}	13.27^{bc}	0.718	0.001
TP (g/L)	61.56	60.89	62.84	59.00	63.21	63.44	61.84	67.23	0.635	0.087
D 120 of the trial										
ALB (g/L)	21.62	23.85	24.29	21.11	19.70	19.96	22.76	23.56	0.460	0.060
ALP (U/L)	347.67^{b}	273.25^{b}	321.00^{b}	502.25^{ab}	647.13^{a}	554.00^{ab}	271.50^{b}	290.29^{b}	35.653	0.021
AST (U/L)	225.17	197.50	179.00	233.38	248.75	251.43	199.88	213.29	7.629	0.173
Ca (mmol/L)	4.94^{cd}	5.42^{bcd}	6.42^{a}	4.84^{d}	4.62^{d}	4.80^{d}	6.11^{ab}	5.74^{abc}	0.125	<0.001
GLU (mmol/L)	10.73	10.05	10.21	11.09	9.74	11.54	10.11	10.66	0.190	0.247
P (mmol/L)	2.01	2.16	2.45	1.97	1.93	1.99	2.22	2.04	0.057	0.282
TG (mmol/L)	9.16	10.64	11.48	9.89	7.88	9.66	11.77	12.12	0.438	0.159
TP (g/L)	57.43	56.03	61.04	55.19	55.04	52.39	55.13	58.66	0.928	0.411

Data are expressed as means with their SEM ($n = 8$). Means within a row without a common superscript letter are significantly different ($P < 0.05$). ALB, albumin; ALP, alkaline phosphatase; AST, aspartate aminotransferase; Ca, calcium; GLU, glucose; P, phosphorus; TG, triglyceride; TP, total protein. Control group was received a basal diet; treatment groups were received a basal diet supplemented with 0.5 % *Leonuri herba* (L group), 0.25 % *Ligustri lucidi fructus* (LF group), 0.25 % *Taraxaci herba* (T group), 0.5 % L + 0.25 % LF (L-LF group), 0.5 % L + 0.25 % T (L-T group), 0.25 % LF + 0.25 % T (LF-T group), and 0.5 % L + 0.25 % LF + 0.25 % T (L-LF-T group), respectively.

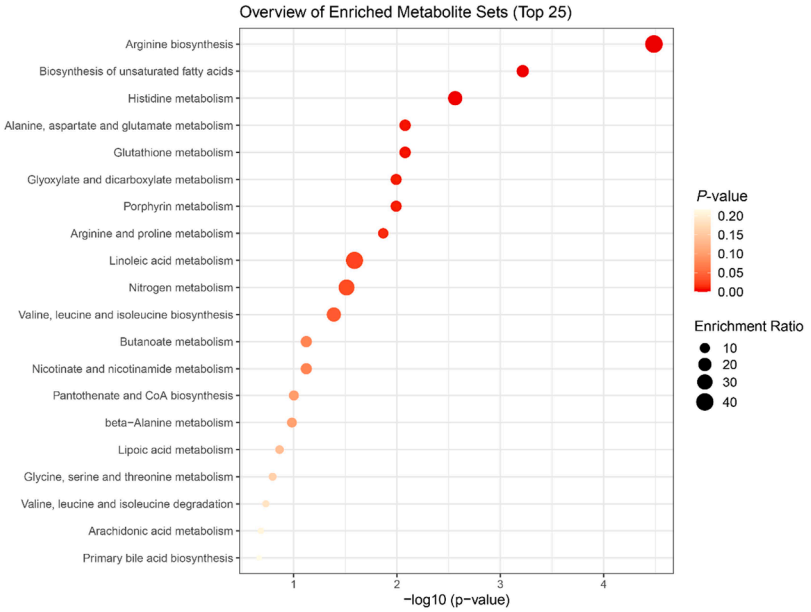

Fig. 3. KEGG pathway enrichment analysis of differential metabolites of fatty acids and amino acids. $P < 0.05$ indicates significantly enriched differential metabolites.

(Ren et al., 2023), whereas AST catalyzes the interconversion of NEAA, such as alanine, aspartate, and glutamate (Racicot et al., 1975). In the present study, dietary L-LF supplementation significantly increased the plasma ALP activity, while LF and LF-T supplementation increased the Ca concentration on d 120 of the trial. However, a previous study concluded that diets supplemented with *Artemisia dracunculus* can reduce blood ALP and AST activities in rainbow trout (Gholamhosseini et al., 2021). In the present study, dietary L, LF, T, and L-LF supplementation reduced the plasma AST activity on d 60 of the trial, indicating that the three individual Chinese herbs significantly contribute to the synthesis of NEAA. This may explain the observed increase in amino acid levels in the present study, including alanine, aspartic acid, and glutamic acid. However, no significant change in AST level during the later stages of the feeding trial may reflect hepatic adaptation to chronic supplementation, warranting further hepatic transcriptomic analysis.

Palmitoleic acid, a MUFA, has the potential to induce metabolic syndrome, diabetes, and inflammation (Guo et al., 2022). In the present study, palmitoleic acid level was negatively correlated with plasma GLU, TP, and TG concentrations. Palmitoleic acid has been reported to enhance glucose homeostasis by reducing hepatic glucose production and enhancing insulin-stimulated glucose uptake in skeletal muscle (Bolsoni-Lopes et al., 2014). In addition, the a* value is an important indicator of the freshness and visual appeal of meat. Palmitoleic acid showed a positive correlation with a* value. Since the oxidation state of myoglobin is closely linked to the meat redness (Su et al., 2024), the oxidation of palmitoleic acid may indirectly influence the a* value by

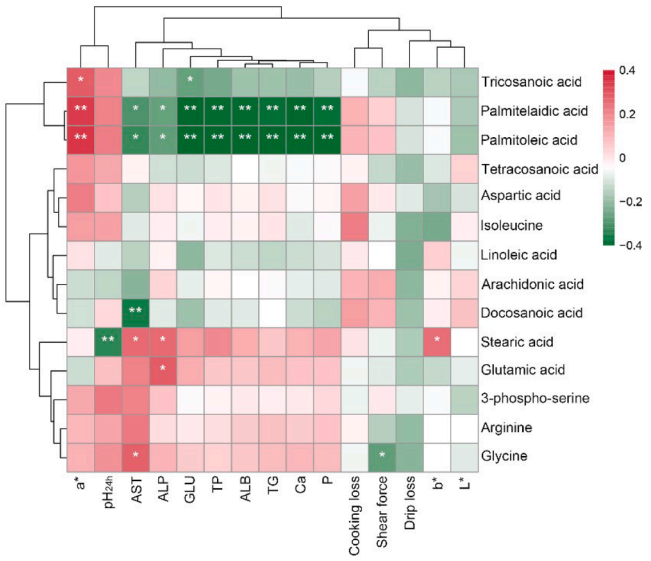

Fig. 4. Correlation heatmap between differential metabolites, meat quality, and plasma biochemical parameters. a*, redness; b*, yellowness; L*, lightness; ALB, albumin; ALP, alkaline phosphatase; AST, aspartate aminotransferase; Ca, calcium; GLU, glucose; P, phosphorus; TG, triglyceride.

altering myoglobin oxidation. This implies that the levels of UFA in meat could serve as a selection criterion to enhance or assess meat quality and the condition of the organism.

To further investigate the key compounds and targets of CHUP that influence meat quality, the targets of compounds derived from herbs, as well as those related to the meat quality of laying hens, were identified through a combination of network pharmacology analysis and molecular docking using multiple databases. The network analysis revealed that linolenic acid and arachidonic acid are the main bioactive compounds associated with L-LF-T, while the key targets affecting meat quality included PPARA, IL6, and HIF1A. These key targets are significant regulators of fatty acid metabolism. PPARA, a major nuclear receptor, plays a critical role in regulating fatty acid oxidation and lipid metabolism (Cui et al., 2024). In addition, this study highlighted core targets within the PPAR signaling pathway. A previous study suggested that upregulation of PPAR signaling pathway and fatty acid metabolism-related targets may be correlated with intramuscular fat deposition and meat quality in Diannan small ears pigs (Fang et al., 2022).

IL6, often considered a pro-inflammatory cytokine, also directly stimulates adipocytes to promote lipolysis, thereby enhancing the release and utilization of fatty acids (Agca and Kir, 2024). Similarly, HIF1α and HIF2α inhibit fatty acid β-oxidation by decreasing PGC-1α (Liu et al., 2014). Additionally, molecular docking simulations demonstrated that linolenic acid and arachidonic acid exhibit strong binding affinity to PPARA, IL6, and HIF1A, forming stable conformations within their respective docking pocket. These findings suggest that linolenic acid and arachidonic acid may modulate fatty acid metabolism by regulating PPARA, IL6, and HIF1A, thereby influencing meat quality in laying hens.

Conclusions

Dietary CHUP supplementation may enhance the concentrations of amino acids and UFA in the pectoral muscle of aged laying hens, potentially by stimulating the PPAR signaling pathway. The beneficial effects of dietary L, L-LF, and L-LF-T supplementation were more pronounced, as these feed additives were associated with increased levels of PUFA, UFA, and amino acids, as well as decreased AI and TI values in the pectoral muscle. This study offers scientific evidence supporting the role of CHUP in improving the meat quality of aged laying hens. Furthermore, this study also establishes a foundation for further investigation into the effective components and mechanisms by which dietary CHUP may enhance meat quality of laying hens. However, additional in-depth research is needed to elucidate the specific practical applications of these feed additives in poultry farming.

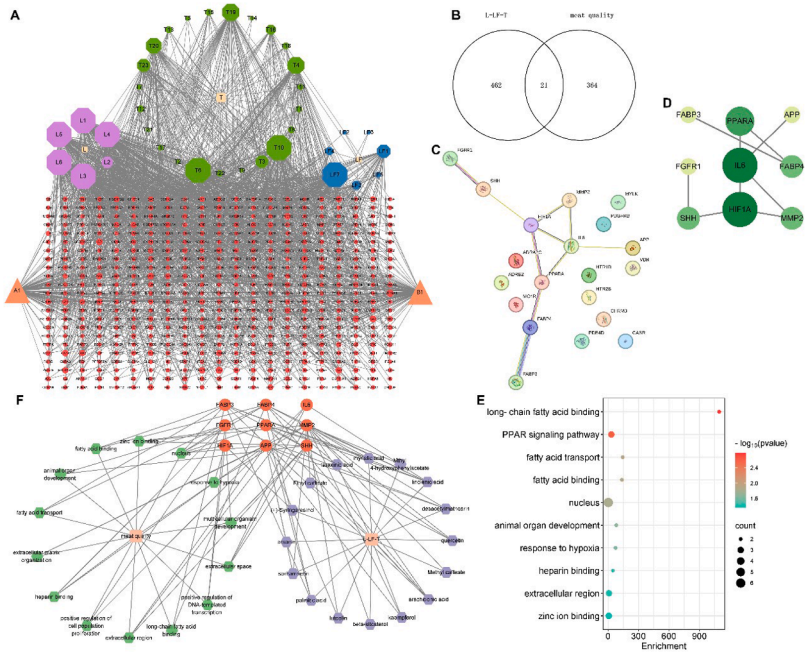

Fig. 5. Network pharmacology analysis of the targets of L-LF-T and meat quality. (A) Herb-compound-target network. (B) 21 intersection targets of L-LF-T and meat quality. (C) STRING interaction network by intersection targets. (D) Core targets of the PPI network screened by intersection targets. (E) Functional enrichment analysis of nine core targets. (F) Drug-compound-target-pathway-meat quality network. L-LF-T, basal diet supplemented with 0.5 % *Leonuri herba* + 0.25 % *Ligustri lucidi fructus* + 0.25 % *Taraxaci herba*.

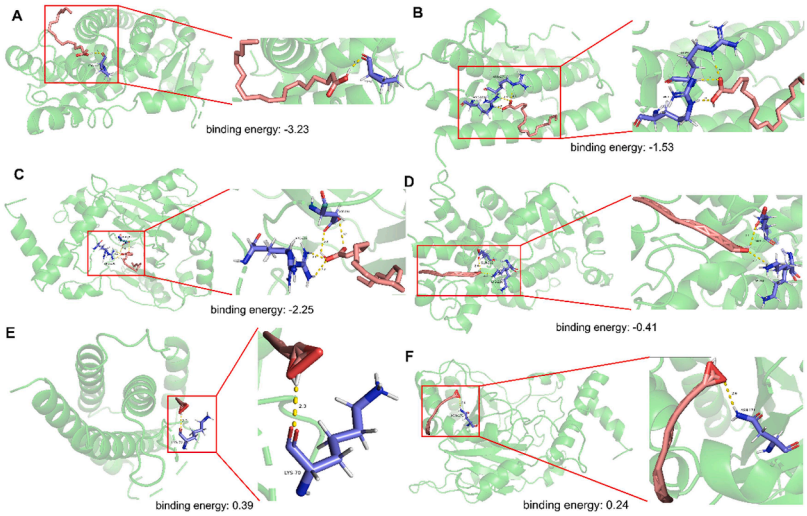

Fig. 6. Molecular docking pattern diagrams between bioactive compounds and core targets. (A–C) Linolenic acid docked into the docking pocket with PPARA, IL6, and HIF1A. (D–F) Arachidonic acid docked into the docking pocket with PPARA, IL6, and HIF1A. Green represents the protein structure; pink represents the binding compound; blue represents the bioactive molecules in the target; yellow dashed lines indicate hydrogen bond interaction forces that facilitate the binding of the molecule to the compound.

Data availability statement

The data presented in this study are deposited in the online repositories, and accession number can be found at 10.57760/sciencedb.16671.

CRediT authorship contribution statement

Jue Gui: Formal analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. **Wenchao Lin:** Formal analysis, Investigation, Methodology, Writing – review & editing. **Chengwen Meng:** Formal analysis, Investigation, Methodology. **Yadong Cui:** Funding acquisition, Writing – review & editing. **Wei Lan:** Investigation, Methodology, Writing – review & editing. **Jianhua He:** Investigation, Methodology, Writing – review & editing. **M.A.K. Azad:** Visualization, Validation, Funding acquisition, Writing – original draft, Writing – review & editing. **Xiangfeng Kong:** Conceptualization, Funding acquisition, Project administration, Supervision, Validation, Writing – review & editing.

Disclosures

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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