scientific reports

Check for updates

OPEN

Dietary wet fermented Brewer's grains modulate hepatic metabolism in pullets

Yuanfeng Li✉, Yiyuan Wang & Zhiheng Meng

This study was conducted to evaluate the effects of wet-fermented brewer's grains (WFBG) on liver metabolism in pullets. A total of 120 female 84-d-old pullets (575.2 ± 4.3 g) were randomly allocated into 2 treatments (0% and 20% WFBG) with 6 replicates per group and 10 birds per replicate in this 28-d experiment. Birds fed 20% WFBG had higher ($P < 0.05$) superoxide dismutase (SOD) level and lower ($P < 0.05$) malondialdehyde (MDA) content in the liver compared with the control group. In total, 324 liver differentially-expressed metabolites (DEMs) including 208 up-regulated DEMs and 116 down-regulated DEMs were identified and considered to be related with WFBG. Pathway analysis revealed that these DEMs were mainly involved in 64 metabolic pathways including metabolic pathways metabolism, glycerophospholipid (GP) metabolism, linoleic acid (LA) metabolism, arachidonic acid metabolism (ARA), and alpha-linolenic acid metabolism. Furthermore, untargeted metabolomic analyses uncovered 18 common up-regulated DEMs and 8 common down-regulated DEMs in GP, LA and ARA metabolism in the 20% WFBG group ($P < 0.05$), such as lysophosphatidylcholine (LPC(18:0/0:0), LPC(0:0/20:4)), lysophosphatidylethanolamine (LPE(22:6/0:0)), and 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine. Overall, the inclusion of 20% WFBG in the diets of pullets led to alterations in liver metabolism.

Keywords Wet-fermented Brewer's grains, Pullet, Metabolomics, Liver health

With the intensification of geopolitical conflicts, the global food crisis is becoming increasingly severe. Using processed non-traditional agricultural and industrial by-products as new raw materials for poultry feed may be a beneficial means to minimize production costs and improve production efficiency[1]. Brewer's grain is the main by-product of the brewing industry, accounting for 85% of the waste and by-products in beer production[2]. Previous studies found that dried brewer's grain was widely used in the diet of laying hens[3], broilers[4,5], quails[6], and ducks[7], which played positive roles in improving the growth performance of poultry[8,9], modulating the balance of gut microbial populations[10], increasing antioxidant capacity[11] and the apparent digestibility of nutrients[4], and reducing breeding costs[12]. Wet brewer's grain (WBG) has also been used in livestock and poultry feed due to its high nutritional value, including dietary fiber, amino acids, and polyphenols[13]. After fermentation, WBG can overcome its inherent drawbacks such as high moisture and crude fiber content, along with low protein and lysine levels[14]. Additionally, it has the potential to enhance the crude protein level, boost the utilization rate of cellulose, and improve palatability[2]. With low cost and moderate nutrition, the large amount of WBG produced in beer production has great potential in animal production. Due to the symbiosis of a large number of microorganisms in the rumen of ruminants, it can ferment dietary fibers to produce volatile fatty acids for energy supply, and can also synthesize proteins and B vitamins[15]. WBG has been widely used in ruminants[15,16], but its application in the poultry industry is relatively scarce[10,17].

Metabolomics can analyze metabolites in biological fluids, cells and tissues, which is commonly used as a tool for biomarker discovery[18,19]. Qualitative, quantitative and differential analysis of metabolites can help in finding solutions and the relationship between metabolites and biological processes[20]. In recent years, metabolomics technology has been widely used to evaluate the fermented feed in pigs[21], laying hens[22], ducks[23], and broilers[24], as well as to identify liver metabolic diseases[25]. So far, there is no existing report regarding the application of metabolomics to investigate the impacts of a WFBG-based diet on liver metabolites in pullets.

In the production of laying hens, fatty liver syndrome is a common nutritional and metabolic disease, caused by long-term fat metabolism disorders. The liver health in laying hens is an important prerequisite for achieving high production performance. The liver plays a critical role in lipid metabolism and the synthesis of yolk precursors, such as vitellogenin and very-low-density lipoproteins (VLDLs), which are essential for egg

College of Agriculture and Biology, Liaocheng University, Liaocheng 252000, China. ✉email: liyuanfeng@lcu.edu.cn

production[26]. Thus, it is hypothesized that WFBG, rich in polyphenols and bioactive compounds, may improve liver function and reduce fat deposition in pullets by modulating lipid metabolism pathways. Therefore, the objective of this study was to investigate the effects of WFBG on liver metabolomics profiles in pullets.

Materials and methods

Animals and treatments

The Luxi green-shell pullets, a native breed of Shandong Province, China, were used in this study. A total of 120 green-shell pullets at 84 d of age were randomly assigned to 2 groups (6 replicates per group and 10 birds per replicate). The control and experimental groups were fed with basic feed or basic feed + additional 20% WFBG (basic feed: WFBG = 80:20), respectively, according to the results of our preliminary experiment (unpublished data). The experiment lasted for 28 d in summer. The birds were purchased from Hongwei Rare Bird Hatchery in Rizhao City, Rizhao, Shandong Province, China. The basic feed of pullets was purchased from a commercial feed enterprise (Haiding Feed Co., Ltd, Liaocheng, China), and the nutritional composition of the feed were shown in Table 1. The experiment protocol (No: LCU20240016) received prior approval from the Animal Protocol Review Committee of Liaocheng University (Liaocheng, Shandong, China). This study was reported in accordance with ARRIVE guidelines, and all methods were performed in accordance with the relevant guidelines and regulations. The WFBG used in this experiment was prepared in the laboratory of Liaocheng University (Liaocheng, Shandong, China) (WBG : corn : wheat bran : fermenting agent = 45:23:30:2, incubated at 37 ℃ for 72 h, once a week). The fermenting agent, mainly consisting of *Lactobacillus acidophilus*, *Bifidobacterium* and *Bacillus subtilis*, was provided by Qingdao Genyuan Biotechnology Group (Qingdao, Shangdong, China).

Sample collection

On d 28, all birds were fasted for 12 h before blood samplings. Six birds closest to the average weight of the pen were randomly selected from each pen of the control and 20% WFBG groups. The birds were euthanized with exsanguination under sodium pentobarbital anesthesia (60 mg·kg^{-1}). Subsequently, the organs and tissues, namely the liver, spleen and kidney, were carefully dissected and weighed. The relative organ weight was calculated and presented as a percentage of the body weight (BW). The liver tissues were carefully removed and snap-frozen in liquid nitrogen, then stored at − 80 °C for metabolomics analysis and further analysis.

Ingredients	CON group	20% WFBG group
Corn, %	50.00	40.00
Wheat, %	15.00	12.00
Wheat bran, %	6.80	5.44
Soybean meal (CP 43%), %	13.80	11.04
Rapeseed meal, %	10.00	8.00
Soybean oil, %	0.60	0.48
WFBG, %	-	20.00
Calcium monophosphate, %	2.50	2.00
Calcium carbonate, %	0.40	0.32
Premix, %[1]	0.50	0.40
NaCl, %	0.40	0.32
Total, %	100.00	100.00
Nutrient levels		
ME, MJ/kg[2]	11.56	11.05
Dry matter, %[3]	88.68	84.28
Crude protein, %[3]	16.85	15.78
Crude fibre, %[3]	4.18	4.75
Crude ash, %[3]	2.95	3.12
Lysine, %[2]	0.60	0.51
Methionine, %[2]	0.30	0.24
Methionine + Cystine, %[2]	0.75	0.60
Threonine, %[2]	0.45	0.36
Calcium, %[3]	3.50	2.90
Available phosphorus, %[2]	0.45	0.38

Table 1. Diet composition (as-fed basis). [1]Provided per kg of diet: Cu, 25 mg; Fe, 80 mg; Zn, 80 mg; Mn, 150 mg; Se, 0.5 mg; I, 0.8 mg. Choline chloride, 1,000 mg; vitamin E, 35 mg; vitamin D_3, 3,000 IU; vitamin A, 10,000 IU; vitamin B_1, 2.5 mg; vitamin B_2, 4 mg; vitamin B_6, 2.5 mg; vitamin K_3, 2 mg; calcium-D-pantothenate, 35 mg; folic acid, 1 mg; nicotinic acid, 50 mg[2]Calculated values[3]Measured values. .

Liver examination

The levels of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), total antioxidant capacity (T-AOC), and malondialdehyde (MDA) in the liver were analyzed using commercial test kits purchased from Nanjing Jiancheng Bio-Engineering Institute (NJBI, Nanjing, China)[27]. The determination of crude fat content in the liver was carried out in accordance with National Standards of the People's Republic of China (GB 5009.6–2016, China)[28].

Liver metabolite extraction

Liver preparation for metabolomics

Liver samples were thawed on ice prior to analysis and vortexed for 10 s for thorough mixing. Liver samples (50 µL) were precisely measured, then 300 µL of acetonitrile/methanol (1:5, v/v) was added. The mixture was adequately vortexed and homogenized for 3 min. Then, the mixture was centrifuged at 12,000 g for 10 min (4 °C). Next, 200 µL of the supernatants were transferred and stored at −20 °C for 30 min, then the mixture was again centrifuged at 12,000 g for 3 min (4 °C). Lastly, 180 µL of the supernatants were injected into the ultra-high liquid chromatography–tandem mass-spectrometry (UHPLC-MS/MS) system for analysis[29]. Quality control (QC) samples pooled from all liver samples were prepared and analyzed with the same procedure.

Metabolomics data capture

Ultraperformance liquid chromatography system was used to carry out chromatographic separation of the liver (LC 30 A, Shimadzu, Japan). The liver was injected on Waters ACQUITY Premier HSS T3 Column (2.1 × 100 mm, 1.8 µm) at 40 °C (0.40 mL/min flow rate). The mobile phase was consisted of A (0.1% formic acid in water) and B (0.1% formic acid in acetonitrile). Further details regarding metabolomic analysis were provided in the Supplementary file 1.

Metabolites identification and pathway analysis

Compound Discoverer 3.0 (Thermo Fisher, USA) was used to convert the raw MS spectra to common data format (.mzML). Candidate metabolites (VIP > 1 and $P < 0.05$) were regarded as potential biomarkers. The metabolite structure was confirmed through LC-MS/MS analysis. The basis of the METLIN were used to search accurate mass value of the metabolites and MS/MS fragment ions. The KEGG[30] (www.kegg.jp/feedback/copyright.html) and HMDB database were used to search metabolic pathways and biochemical reactions. Pathway analysis and visualization were performed on Compound Discoverer 3.0 software (Thermo Fisher Scientific, USA).

Statistical analysis

Data was analyzed by Student's *t*-test of SPSS 23.0 (SPSS Inc., Chicago, Illinois, USA)[31]. All data were expressed as the mean. Variability in the data was expressed as the standard error of the means (SEM). *P*-values < 0.05 were considered significant.

Results

Liver profiles

The relative weight of the liver in the 20% WFBG group was significantly lower than that in the control group ($P < 0.05$, Table 2). Additionally, hepatic fat content in the 20% WFBG group was significantly lower than that in the control group ($P < 0.05$, Table 2).

Compared with the control group, the 20% WFBG group exhibited significantly higher hepatic SOD and GSH-Px activities ($P < 0.05$, Table 3), whereas hepatic MDA content was significantly lower ($P < 0.05$, Table 3).

Liver untargeted metabolomics profile of WFBG

Quality control of metabolite data from liver samples

2D-PCA was performed on all samples in the study (Fig. 1A). In the PCA score plots, the liver samples of pullets in the 20% WFBG group showed a tendency away from the control group, indicating significant changes in liver metabolic profiles of pullets fed with WFBG diet. Then, PLS-DA was performed to identify the potential metabolites that were different between the control and 20% WFBG groups. Clear separate clustering patterns between the control and 20% WFBG groups were found by PLS-DA score plots (R2 X = 0.289, R2 Y = 0.981, Q2 = 0.620) (Fig. 1B). In PLS-DA score plots, 20% WFBG group showed a trend to be away from the control group, indicating the distinguishable changes in the liver from the WFBG group at the metabolite level. Two hundred random permutation tests were applied to guard against OPLS-DA model overfitting (Fig. 1C). Results of the permutation test showed that the intercepts were R2 X = 0.383, R2 Y = 0.998, and Q2 = 0.478) (Fig. 1C), which indicated that the OPLS-DA models were robust without overfitting.

Items	CON group[2]	20% WFBG group[2]	SEM[3]	*P*-values
Liver, %	2.52[a]	2.31[b]	0.09	0.039
Liver fat content, %	1.87[a]	1.72[b]	0.04	0.004

Table 2. Effects of dietary WFBG on organ index and liver fat content in pullets[1]. [1]Means represent 6 replicates with 10 birds per cage ($n = 6$/group). . [2]CON group: basal diet; 20% WFBG group: basal diet + 20% WFBG. . [3]Pooled standard error of mean. . WFBG, wet-fermented brewer's grains. Values within a row with different superscripts differ significantly at $P < 0.05$.

Items[2]	CON group[3]	20% WFBG group[3]	SEM[4]	*P*-value
SOD, IU/mg	444.58[b]	601.63[a]	41.59	0.004
GSH-Px, IU/mg	183.38[b]	226.95[a]	13.28	0.016
T-AOC, IU/mg	0.99	0.95	0.04	0.336
MDA, nmol/mg	2.27[a]	1.86[b]	0.10	0.002

Table 3. Effects of dietary WFBG on the liver antioxidant capacity in pullets[1]. [1]Means represent 6 replicates with 10 birds per cage ($n = 6$/group). . [2]GSH-Px, glutathione peroxide; T-AOC, total antioxidant capacity; MDA, malondialdehyde. . [3]CON group: basal diet; 20% WFBG group: basal diet + 20% WFBG. . [4]Pooled standard error of mean. . WFBG, wet-fermented brewer's grains. Values within a row with different superscripts differ significantly at $P < 0.05$.

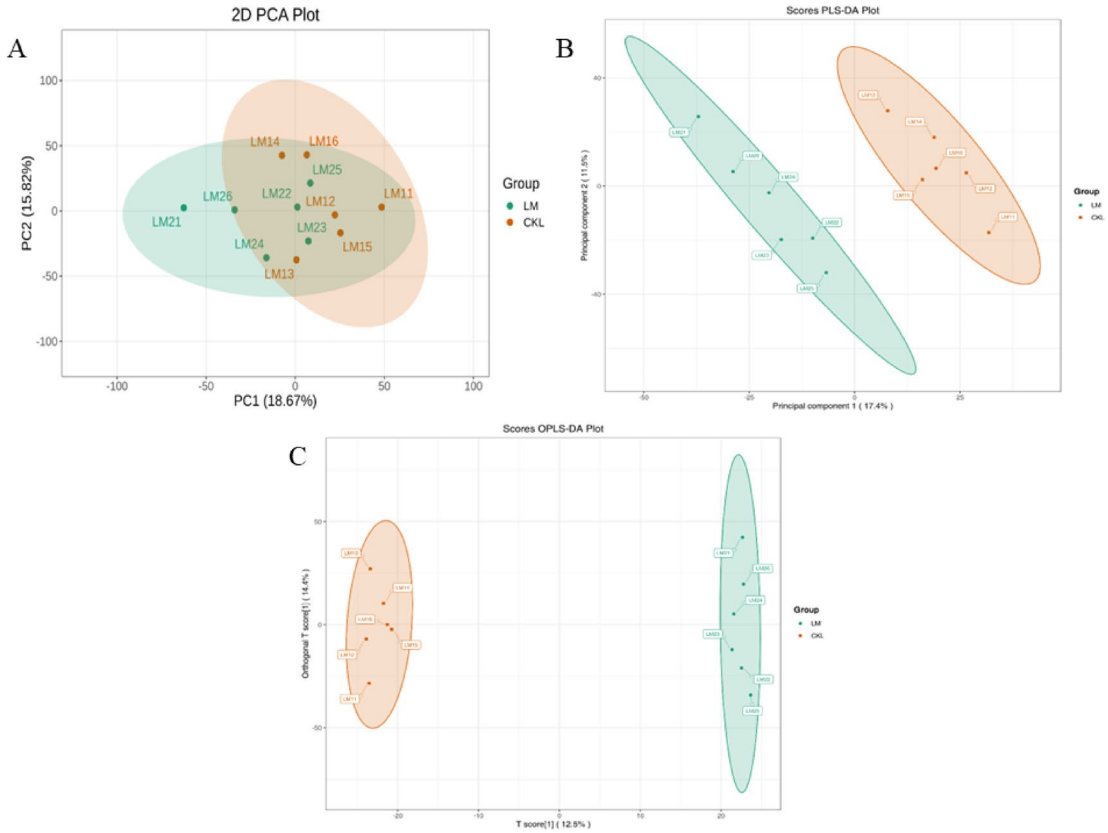

Fig. 1. Liver metabolome analysis. (**A**) PCA-DA score plots derived from UHPLC-MS/MS. PC1 represents the first principal component, PC2 represents the second principal component, and the percentage represents the explanatory power of the principal component on the dataset; Each point in the figure represents a sample, samples in the same group are represented by the same color. (**B**) PLS-DA of the liver metabolites from the control and 20% WFBG groups (R2 X = 0.289, R2 Y = 0.981, Q2 = 0.620). (**C**) OPLS-DA of the liver metabolites from the control and 20% WFBG groups (R2 X = 0.383, R2 Y = 0.998, and Q2 = 0.478). R2 X and R2 Y indicate the fraction of the variables explained by the model, and the Q2 shows the predictive abilities of the model.

Differential metabolite analysis

According to the relative abundance of liver metabolites, hierarchical clustering analysis was performed to generate a heatmap to show a global view of the metabolites (Fig. 2). The screening criteria used to identify differential metabolites in the liver of pullets from the 20% WFBG and control groups were VIP score > 1, Fold Change (FC) > 1.2, or FC < 0.833, and adjusted *P*-value < 0.05. There were 324 differentially-expressed metabolites (DEMs) in the liver, including 208 up-regulated DEMs and 116 down-regulated DEMs in the 20% WFBG group compared with the control group (Fig. 3A). Among these DEMs, the levels of hellebrin, 1-(9Z-heptadecenoyl)-sn-glycero-3-phosphoethanolamine, Lys-Pro-Tyr, carnitine C12:1, glycine linoleate, 2'-deoxycytidine 3'-monophosphate, D-mannonic acid, among others, were increased in the 20% WFBG group, and the levels of 2-methylpropane-1,2-diol, cholest-4-en-3-one, PE-NMe(15:0/22:4(7Z,10Z,13Z,16Z)), N-valylphenylalanine, asparagylaspartic acid, diethyltoluamide, D-homocysteine, 4-methylvaleric acid, among

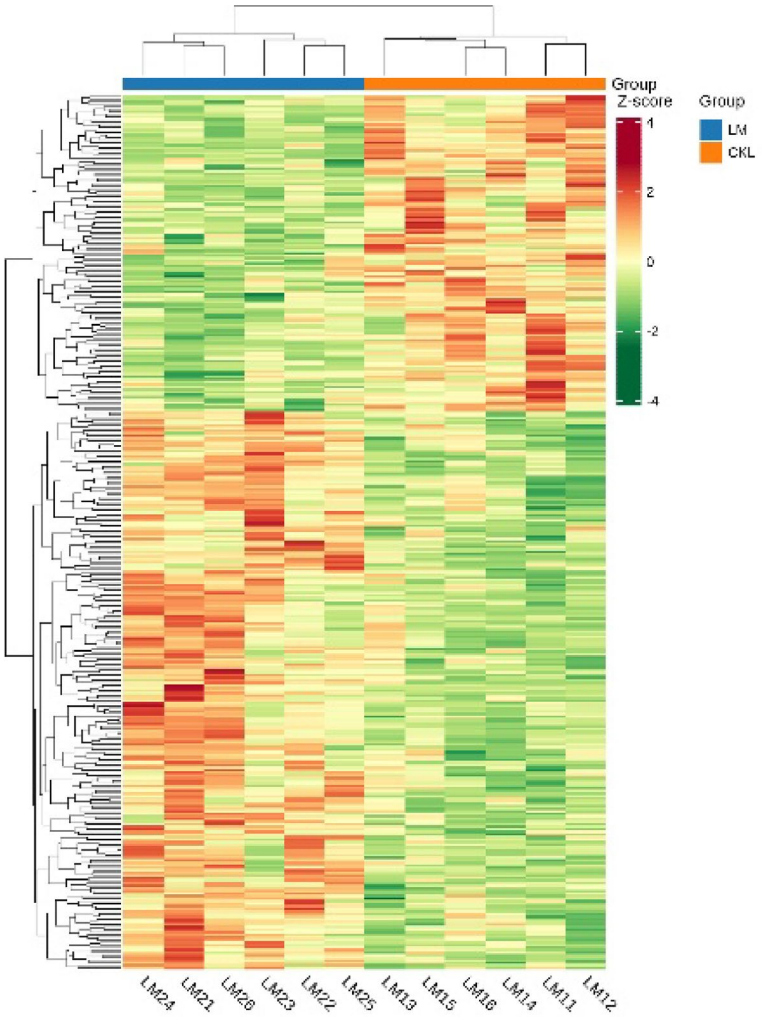

Fig. 2. Hierarchical cluster analysis. Heat map representation of metabolites that differed significantly between liver samples of two groups. Each block represents the abundance of one metabolite from one sample. CKL control group; LM, 20% WFBG group.

others, were decreased. The top-10 up-regulated and down-regulated DEMs were shown in Table 4. More detailed results were shown in the Supplementary Table 1.

KEGG pathway analysis

A total of 5715 metabolites identified in both positive and negative ion modes were subjected to KEGG pathway analysis. The key functional effects of enriched DEMs in metabolic pathways were analyzed using MetaboAnalyst (Fig. 3B). The enriched DEMs between the 20% WFBG and control groups had a significant effect on 64 metabolic pathways, such as metabolic pathways, glycerophospholipid (GP) metabolism, linoleic acid (LA) metabolism, arachidonic acid (ARA) metabolism, alpha-linolenic acid metabolism, autophagy-animal, glycerolipid metabolism, and ABC transporters (adjusted $P < 0.05$). There were 78 metabolites involved in metabolic pathways, 46 metabolites involved in GP metabolism, 27 metabolites involved in LA metabolism, 26 metabolites involved in ARA metabolism, 26 metabolites involved in alpha-linolenic acid metabolism, 13 metabolites involved in autophagy-animal, 9 metabolites in glycerolipid metabolism, and 6 metabolites in ABC transporters. Therefore, we further investigated the changes of GP, LA and ARA metabolites in the liver of pullets fed 20% WFBG diet. Summary of the results of metabolic pathway analysis was showed in the Supplementary Table 2.

GP, LA and ARA untargeted metabolomic studies

Results of quantitative analysis of 46 GP metabolites were shown in Fig. 4A. Compared with the control group, it was found that the levels of 27 DEMs (LPE(22:6/0:0), glycerophosphoethanolamine, LPC(18:0/0:0), LPC(0:0/20:4), and 1,2-dioleoyl-sn-glycero-3-phosphocholine, etc.) were markedly increased in the 20% WFBG group ($P < 0.05$), and the levels of 19 DEMs (1-stearoyl-2-linoleoyl-sn-glycero-3-phosphoethanolamine, PE-NMe(15:0/20:3(5Z,8Z,11Z)), PE-NMe2(16:0/18:2(9Z,12Z)), PE-NMe2(14:1(9Z)/20:0), 1 sn-glycerol 3-phosphate, 11-stearoyl-2-linoleoyl-sn-glycerol, choline, etc.) were markedly decreased in the 20% WFBG

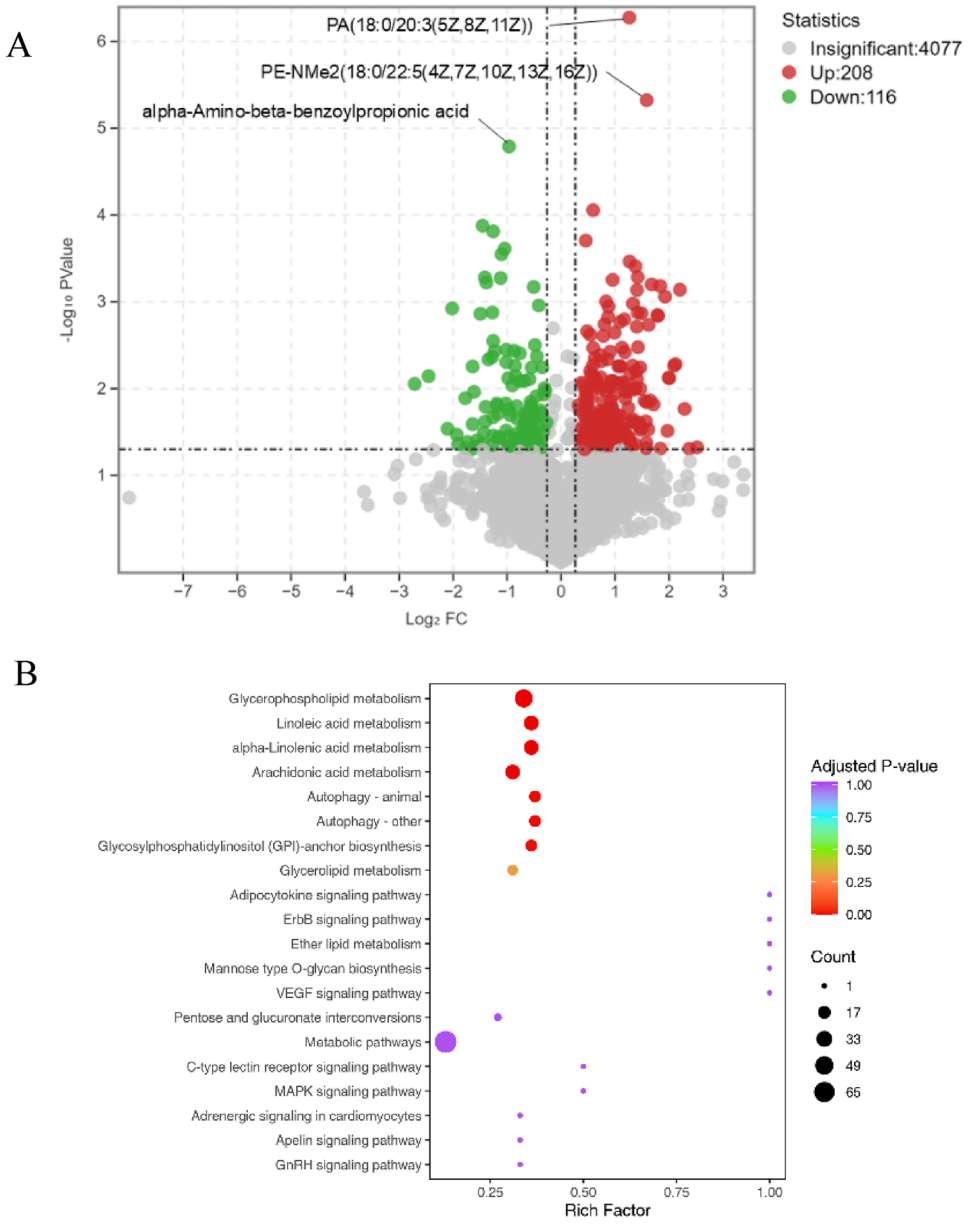

Fig. 3. Volcanic map and pathway analysis of identified liver metabolites related to WFBG. (**A**) Volcanic map in the control and 20% WFBG groups (adjusted *P*-value < 0.05 and Fold Change (FC) > 1.2, or FC < 0.833). Volcano plot representing the significant variables in the discrimination of liver metabolites from two groups. Gray, unchanged metabolites. Red, up-regulated metabolites. Green, down-regulated metabolites. (**B**) The KEGG pathways of DEMs from the control and 20% WFBG group. Advanced bubble chart shows the enrichment of DEMs in signaling pathways. The x-axis represents the rich factor (rich factor = number of DEMs enriched in the pathway/number of all metabolites in the background metabolites set). The y-axis represents the enriched pathway. Size of the bubble represents the number of DEMs enriched in the pathway, and the color represents enrichment significance.

group ($P < 0.05$). Results of quantitative analysis of 27 LA metabolites were shown in Fig. 4B. Compared with the control group, it was found that the levels of 19 DEMs (bovinic acid, Pov-pc, LPC(22:6/0:0), butenoyl-PAF, and PC(16:0/2:0), etc.) were markedly increased in the 20% WFBG group ($P < 0.05$), and the levels of 8 DEMs (1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine, PC(20:3(5Z,8Z,11Z)/24:1(15Z)), 1-docosanoyl-2-pentadecanoyl-glycero-3-phosphocholine, etc.) were markedly decreased in the 20% WFBG group ($P < 0.05$). Results of quantitative analysis of 26 ARA metabolites were shown in Fig. 4C. Compared with the control group, it was found that the levels of 18 DEMs (1,2-dimyristoleoyl-sn-glycero-3-phosphocholine, 1,2-dipentadecanoyl-sn-glycero-3-phosphocholine, PC(20:4(5Z,8Z,11Z,14Z)/18:0), etc.) were markedly increased in the 20% WFBG group ($P < 0.05$), and the levels of 8 DEMs in the 20% WFBG group were the same as those of ARA metabolism, and significantly decreased ($P < 0.05$). In addition, there were 26 common DEMs (18 up-regulated DEMs and 8

NO.	Metabolite	Adduct	RT(s)	VIP	FC	Mode	Adjusted *P*-value
1	Hellebrin	[M + Na]+	4.17	1.81	1.44	up	0.0246
2	1-(9Z-heptadecenoyl)-sn-glycero-3-phosphoethanolamine	[M + NH4]+	7.43	1.61	1.00	up	0.0459
3	Lys-Pro-Tyr	[M + NH4]+	7.33	2.04	1.19	up	0.0130
4	[(1 S,7 S,8 S,8aR)−8-[2-[(2R,4R)−4-hydroxy-6-oxo-tetrahydropyran-2-yl]ethyl]−7-methyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl] (2 S)−2-methylbutanoate	[M + Na]+	2.71	1.80	0.83	up	0.0438
5	Parecoxib	[M + H]+	1.44	1.76	0.53	up	0.0345
6	Carnitine C12:1	[M+]+	5.45	1.70	0.86	up	0.0299
7	Glycine linoleate	[M-H]-	7.62	1.83	0.76	up	0.0198
8	Ser-Pro-Ile	[M + H]+	2.51	2.04	1.71	up	0.0152
9	2'-deoxycytidine 3'-monophosphate	[2 M-H]-	1.88	1.90	0.91	up	0.0423
10	D-Mannonic acid	[2 M-H]-	0.56	1.73	0.57	up	0.0455
11	2-Methylpropane-1,2-diol	[M-H]-	0.99	1.96	0.89	down	0.0212
12	PC(18:2(9Z,12Z)/22:6(4Z,7Z,10Z,13Z,16Z,19Z))	[M + CH3 COO]-	8.94	2.06	0.82	down	0.0107
13	PE-NMe(15:0/22:4(7Z,10Z,13Z,16Z))	[M-H2O-H]-	8.94	1.77	0.83	down	0.0247
14	Cholest-4-en-3-one	[M + Cl + NH3]-	6.68	1.83	0.86	down	0.0257
15	N-Valylphenylalanine	[M + CH3 COO]-	2.77	2.03	0.94	down	0.0081
16	Asparagylaspartic acid	[M+]+	9.13	2.24	0.90	down	0.0020
17	Altretamine	[M + H]+	5.94	1.96	0.92	down	0.0163
18	Diethyltoluamide	[M + H]+	5.94	1.97	0.86	down	0.0322
19	D-Homocysteine	[M + NH4]+	1.39	2.16	0.81	down	0.0099
20	4-Methylvaleric Acid	[M + H-2H2O]+	4.29	2.03	0.92	down	0.0138

Table 4. Identification results of top-10 DEMs in the liver between the 20% WFBG and control groups. Abbreviations: *FC*, fold change, 20% WFBG group vs. control group; *WFBG*, wet- fermented brewer's grains; *PE*, phosphatidylethanolamine; *RT*, retention time; *VIP*, variable importance in the projection.

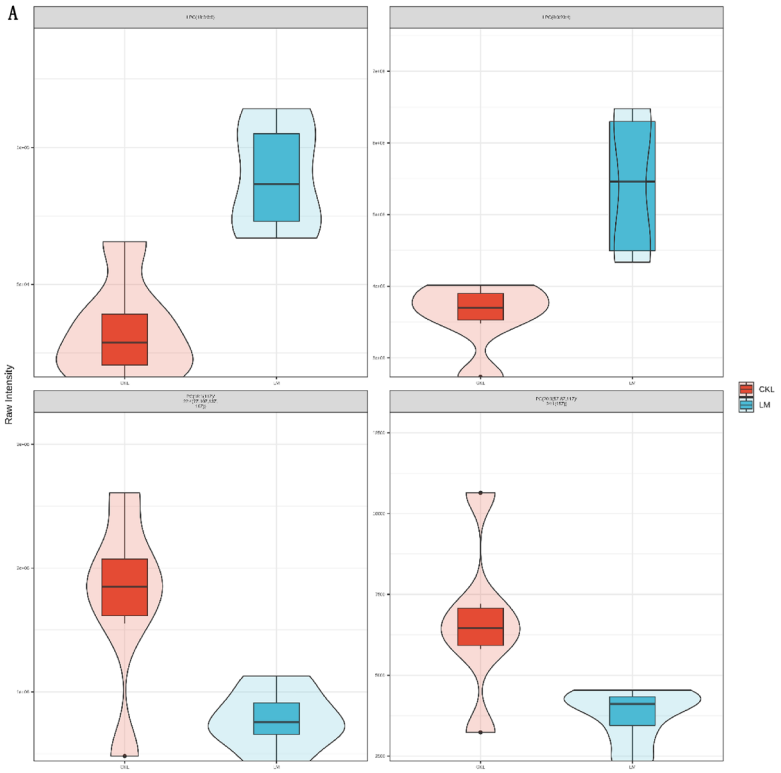

Fig. 4. Concentrations of liver metabolites involved in GP metabolism (**A**), LA metabolism (**B**), and ARA metabolism (**C**) by untargeted analysis. Abbreviations: GP, Glycerophosphate, ARA, arachidonic acid. * $P < 0.05$, compared with the control group. CKL, control group; LM, 20% WFBG group.

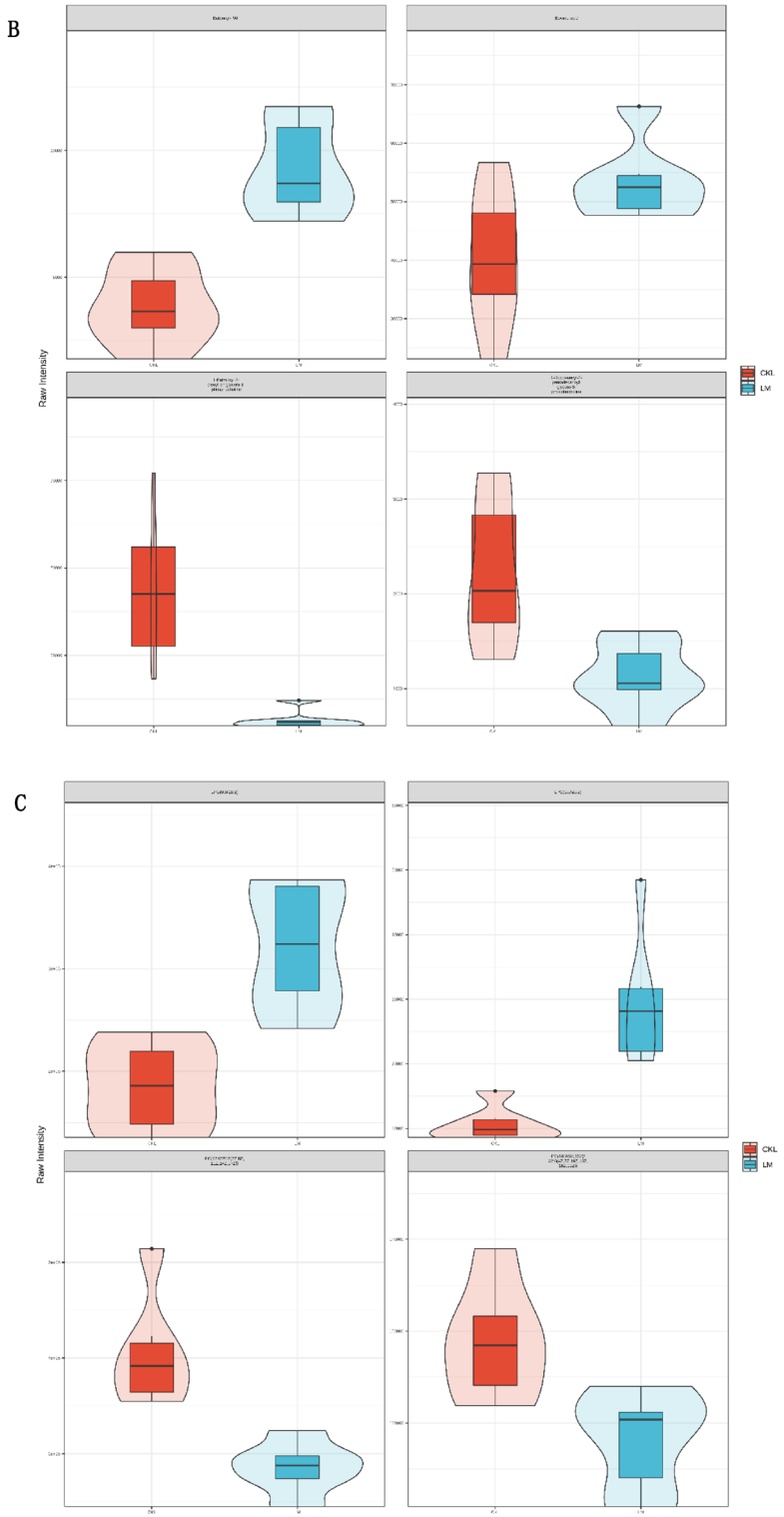

Figure 4. (continued)

down-regulated DEMs) among the GP, LA and ARA metabolism pathways. Summary of the results of metabolic pathway analysis was showed in the Supplementary Table 2.

Discussion

Liver profiles

Under normal circumstances, the antioxidant system in animals is usually in dynamic equilibrium. When external factors stimulate and disrupt this dynamic equilibrium state, it can be disrupted, leading to oxidative stress in

animals[32,33]. Oxidative stress produces generous intermediates such as ROS and reactive nitrogen, which can lead to physical damage[34]. The oxidative stress state of animals can be characterized by the activity of antioxidant enzymes such as SOD, GSH Px, T-AOC, and MDA. Qamar et al. reported that radical scavenging activity and flavonoid and polyphenol content were significantly increased in the FBG, indicating an improvement in antioxidant capacity[11]. In this study, the increase in the SOD and GSH-Px levels and the decrease in the MDA content indicated that 20% WFBG diet enhanced the antioxidant capacity of pullet liver, which was consistent with the changes in liver enzyme activities in Chahua chickens fed with 15% fermented feed[35]. The consistency indicated that fermented feed could improve the antioxidant function of poultry liver. The reduction in liver fat content and liver organ index further substantiated that the inclusion of WFBG in the diet could enhance the hepatic function of pullets and contribute to the amelioration of liver health. Although adding WFBG to the diet may trigger the effect of nutrient dilution, which in turn has potential impacts on the health and growth of animals, the data from previous studies show that a 20% addition dosage can significantly improve the growth performance of pullets. This phenomenon implies that the growth-promoting effect of WFBG is sufficient to offset the negative effects caused by nutrient dilution. Based on this important discovery, we have delved into the metabolic characteristics of the pullet liver, and further revealed the potential molecular mechanisms and metabolic pathways of the growth-promoting effect of WFBG.

Liver untargeted metabolomics profile of WFBG

With the application of WFBG diet in pullets, untargeted UHPLC-MS/MS metabolomics study of liver was performed to investigate promoting growth mechanism of WFBG. The results found that the metabolic profile of liver in the 20% WFBG group deviated from the control group, which suggested the significant changes of liver metabolites in the layers with WFBG. Especially 324 biomarkers such as LPC(18:0/0:0), LPC(0:0/20:4), LPC(22:6/0:0), PE(18:0/20:4(5Z,8Z, 11Z,14Z)), PE-NMe(15:0/22:0), and glycine linoleate associated with WFBG were identified. Pathway analysis showed that these metabolites were mainly involved in metabolic pathways, GP metabolism, LA metabolism, ARA metabolism, alpha-linolenic acid metabolism, autophagy-animal, glycerolipid metabolism, and ABC transporters. Changes of these liver metabolites and the altered metabolic pathways may provide new evidence to understand the action pathway of WFBG.

Lipids, the main components of cell membranes, directly maintain the physio logical functions of cells and participate in the transport of triglycerides[36]. Growing evidence has shown that animal growth is associated with alteration of lipid metabolism[37–39]. Glycerophospholipids are the main components of cell membranes and have garnered considerable attention due to its involvement in a wide array of key biological functions[40]. They directly maintain the physiological functions of cells and participate in the transport of triglycerides[40]. Jia et al. reported that phosphatidylcholine could ameliorate lipid accumulation and liver injury in high-fat diet mice[41]. It has been reported that liver metabolites and GP metabolism were linked to the pathophysiology of potentially alleviate BPA-induced murine liver injury[42]. In the present study, increased DEMs (LPC(18:0/0:0), LPC(0:0/20:4), LPC(22:6/0:0)) and decreased DEMs (1-Palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine, 2-(((R)−2,3-Dihydroxypropyl)phosphoryloxy)-N, N,N-trimethylethanaminium and choline) involved in GP metabolism were observed in the liver from the 20% WFBG group, which might indicate the alteration in GP metabolism. LPC plays a crucial role in the absorption, transport and metabolism of lipids[40]. In the metabolic process of high-density lipoprotein (HDL), LPC can serve as an important lipid component, helping HDL complete the reverse transport of cholesterol, that is, transporting cholesterol from peripheral tissues back to the liver for metabolism, which is of great significance for maintaining the dynamic balance of cholesterol in the body[43]. Increased levels of LPC(18:0/0:0), LPC(0:0/20:4), LPC(22:6/0:0) were observed in the liver from WFBG group, which might indicate that GP metabolism reduced the potential risk of excessive fat deposition in pullets.

Besides GP metabolites, the changed phosphatidylcholines in this study also involved in LA and ARA metabolism pathway. LA, n-6 polyunsaturated fatty acids, plays a key role in inhibiting fatty acid synthesis by promoting the oxidation of saturated fatty acids, thereby reducing the production of cholesterol and triglycerides, and ultimately lowering blood lipids[44]. Linoleic acid deficiency will easily cause disorder of lipid metabolism in the blood, which will increase the risk of atherosclerosis. LA can also alleviate acute lung injury[45] and exhibit pro-tumorigenic or anti-tumorigenic effects in various types of cancer[46]. LA metabolism is an important metabolic pathway involving many important physiological functions, including the oxidation of fatty acids to LA, activating glucose and lipid metabolism processes to maintain blood glucose stability and promote cellular metabolic activity. ARA, n-6 polyunsaturated fatty acids, plays an important role in regulating lipid metabolism, biological processes, immune responses, and disease progressions[47–49]. Ma et al. reported that ARA alleviated cholestatic liver diseases by regulating hepatocyte bile acid uptake[50]. It has been reported that liver metabolites and ARA metabolism were linked to the pathophysiology of fatty liver hemorrhagic syndrome (FLHS) in laying hens[37] and nonalcoholic fatty liver disease[51]. In this study, pathway analysis revealed that linoleic acid (LA) and arachidonic acid (ARA) metabolism were disrupted in pullets fed 20% FBG. Metabolites involved in increased 1,2-dimyristoleoyl-sn-glycero-3-phosphocholine, 1,2-dipentadecanoyl-sn-glycero-3-phosphocholine, PC(20:4(5Z,8Z,11Z,14Z)/18:0), or decreased 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine, 1-docosanoyl-2-pentadecanoyl-glycero-3-phosphocholine, and PC(18:1(11Z)/22:4(7Z,10Z,13Z,16Z)).

In this study, among pullets fed a diet containing 20% WFBG, there were 18 commonly up-regulated DEMs and 8 commonly down-regulated DEMs in in GP, LA and ARA metabolism. In addition, up-regulated bovinic acid were found in LA metabolism. A total of 20 DEMs closely related to GP metabolism, such as LPE(22:6/0:0), glycerophosphoethanolamine, sn-glycerol 3-phosphate, and choline, were also detected. These findings indicated that GP, LA and ARA metabolism represented crucial metabolic pathways underlying the alterations in lipid metabolism of pullets induced by WFBG. Moreover, the inclusion of 20% WFBG in the pullets' diet potentially exerted a positive influence on the changes in lipid metabolism. These DEMs might be potential biomarkers for WFBG to promote the growth of pullets. More animal experiments are needed for further verification.

Conclusions

In summary, untargeted metabolomics analysis showed that 324 DEMs were significantly enriched in 64 metabolic pathways. The changes in liver metabolites and GP, LA and ARA derivatives were linked to the progression of WFBG, which provided a theoretical basis for studying the growth promoting mechanism of pullets at the metabolome level.

Data availability

Data is provided within the manuscript or supplementary files.

Received: 5 February 2025; Accepted: 8 May 2025
Published online: 16 May 2025

References

1. Vlaicu, P. A., Untea, A. E., Varzaru, I., Saracila, M. & Oancea, A. G. Designing nutrition for health-incorporating dietary by-products into poultry feeds to create functional foods with insights into health benefits, risks, bioactive compounds, food component functionality and safety regulations. *Foods* **12** (21), 4001. https://doi.org/10.3390/foods12214001 (2023).
2. Eliopoulos, C., Arapoglou, D., Chorianopoulos, N., Markou, G., Haroutounian, S. A. & Conversion of brewers' spent grain into proteinaceous animal feed using solid state fermentation. *Environ. Sci. Pollut Res.* **29** (20), 29562–29569. https://doi.org/10.1007/s11356-021-15495-w (2022).
3. Deltoro López, J., Fernandez Carmona, J. & Martinez Pascual, J. L. Evaluation of Brewer's dried grains in the diets of laying hens. *Anim. Feed Sci. Technol.* **6** (2), 169–178 (1981).
4. Pfaff, W. K., Moreng, R. E. & Kienholz, E. W. The utilization of brewers' dried grains in the diets of Chinese ringneck pheasant-breeder hens. *Poult. Sci.* **69** (9), 1491–1495. https://doi.org/10.3382/ps.0691491 (1990).
5. Pires Filho, I. C. et al. Effects of feeding dried Brewer's grains to slow-growing broiler chickens. *Live Sci.* **250**, 104561. https://doi.org/10.1016/j.livsci.2021.104561 (2021).
6. Sutton, C. D., Muir, W. M. & Begin, J. J. Effect of fiber on cholesterol metabolism in the Coturnix quail. *Poult. Sci.* **60** (4), 812–817. https://doi.org/10.3382/ps.0600812 (1981).
7. Rooke, J. A., Duthie, C. A., Hyslop, J. J., Morgan, C. A. & Waterhouse, T. The effects on cow performance and calf birth and weaning weight of replacing grass silage with Brewer's grains in a barley straw diet offered to pregnant beef cows of two different breeds. *J. Anim. Physiol. Anim. Nutr.* **100** (4), 629–636. https://doi.org/10.1111/jpn.12410 (2016).
8. Al-Khalaifah, H. S. et al. Effects of graded levels of microbial fermented or enzymatically treated dried Brewer's grains on growth, digestive and nutrient transporter genes expression and cost effectiveness in broiler chickens. *BMC Vet. Res.* **16** (1), 424. https://doi.org/10.1186/s12917-020-02603-0 (2020).
9. Wang, Y. R. et al. Effects of dried brewer' s grain on performance, liver biochemical indices and Gastrointestinal tract development of Jianchang ducks aged from 31 to 59 days. *J. Anim. Nutr.* **28** (3), 807–815 (2016).
10. Lao, E. J., Dimoso, N., Raymond, J. & Mbega, E. R. The prebiotic potential of brewers' spent grain on livestock's health: a review. *Trop. Anim. Health Prod.* **52** (2), 461–472. https://doi.org/10.1007/s11250-019-02120-9 (2020).
11. Qamar, H. et al. Integrated metabolomics and metagenomics unveiled biomarkers of antioxidant potential in fermented Brewer's grains. *Antioxid. (Basel).* **13** (7), 872. https://doi.org/10.3390/antiox13070872 (2024).
12. Tiwari, U. P., Mattus, S., Neupane, K. & Jha, R. In vitro fermentation characteristics of agricultural products and coproducts and its effect on the large intestinal microbiota of swine. *J. Anim. Sci.* **94** (5), 215. https://doi.org/10.2527/jam2016-0450 (2016).
13. Zhang, Y. et al. Applicability of wheat Brewer's spent grain in steamed bread-making based on physicochemical and visual profiles assessment of doughs and breads. *Food Sci. Technol. Int.* **6**, 10820132241260453. https://doi.org/10.1177/10820132241260453 (2024).
14. Kratzer, R. H. Leslie Earl. The feeding value of the protein of Brewer's dried grains for chicks. *Poult. Sci.* **59** (10), 2361–2364 (1980).
15. Moriel, P. et al. Frequency of wet Brewer's grains supplementation during late gestation of beef cows and its effects on offspring postnatal growth and immunity. *J. Anim. Sci.* **94** (6), 2553–2563. https://doi.org/10.2527/jas.2016-0427 (2016).
16. Hatungimana, E., Stahl, T. C. & Erickson, P. S. Effect of storage of wet Brewer's grains with incremental levels of salt on apparent total tract nutrient digestibility and purine derivative excretion in dairy heifers. *J. Anim. Sci.* **99** (1), skaa393. https://doi.org/10.1093/jas/skaa393 (2021).
17. McCarthy, A. L., O'Callaghan, Y. C., Piggott, C. O., FitzGerald, R. J. & O'Brien, N. M. Brewers' spent grain; bioactivity of phenolic component, its role in animal nutrition and potential for incorporation in functional foods: a review. *Proc. Nutr. Soc.* **72(1)**, 117–125. https://doi.org/10.1017/S0029665112002820 (2013).
18. Chai, W. et al. Liquid chromatography-mass spectrometry-based metabolomics reveals dynamic metabolite changes during early postmortem aging of Donkey meat. *Foods* **13** (10), 1466. https://doi.org/10.3390/foods13101466 (2024).
19. Chen, X. et al. LC-MS-based metabolomics reveals metabolite dynamic changes of beef after superchilling early post-mortem. *Food Res. Int.* 183114208. https://doi.org/10.1016/j.foodres.2024.114208 (2024).
20. Khan, M. Z. et al. A review of genetic resources and trends of omics applications in Donkey research: focus on China. *Front. Vet. Sci.* **11**, 1366128. https://doi.org/10.3389/fvets.2024.1366128 (2024).
21. Huang, L. L. et al. Effect of fermented feed on growth performance, holistic metabolism and fecal microbiota in weanling piglets. *Anim. Feed Sci. Technol.* **266**, 114505. https://doi.org/10.2527/jam2016-0450 (2020).
22. Tian, Y. et al. Microbial fermented feed affects flavor amino acids and yolk trimethylamine of Duck eggs via cecal microbiota-yolk metabolites crosstalk. *Food Chem.* **430**, 137008. https://doi.org/10.1016/j.foodchem.2023.137008 (2024).
23. Yang, Q. et al. Untargeted metabolomics study on the effects of rearing ducks in cages on bone quality. *Poult. Sci.* **101** (2), 101604. https://doi.org/10.1016/j.psj.2021.101604 (2022).
24. Qin, J. et al. Gas chromatography-mass spectrometry-based untargeted metabolomics analysis reveals Circulating biomarkers related to wooden breast myopathy in broilers: a preliminary study. *Poult. Sci.* **102** (7), 102718. https://doi.org/10.1016/j.psj.2023.102718 (2023).
25. Tang, J., Xiong, K., Zhang, T. & Han, H. Application of metabolomics in diagnosis and treatment of chronic liver diseases. *Crit. Rev. Anal. Chem.* **52** (5), 906–916. https://doi.org/10.1080/10408347.2020.1842172 (2022).
26. Li, H. et al. Transcriptome profile of liver at different physiological stages reveals potential mode for lipid metabolism in laying hens. *BMC Genom.* **16**, 763. https://doi.org/10.1186/s12864-015-1943-0 (2015).
27. Li, Q., Yang, S., Chen, F., Guan, W. & Zhang, S. Nutritional strategies to alleviate oxidative stress in sows. *Anim. Nutr.* **9**, 60–73. https://doi.org/10.1016/j.aninu.2021.10.006 (2021).
28. National Standards of the People's Republic of China Determination of fat in food. In *GB 5009.6–2016* (National Health and Family Planning Commission of the People's Republic of China and State Food and Drug Administration, 2016).
29. Li, Y., Tao, X., Zhao, P., Zhou, J. & Ao, X. Effects of slaughter weight on carcass characteristics, meat quality, and metabolomics profiling in the longissimus dorsi muscle of Tianfu finishing pigs. *Front. Veter Sci.* **11**, 1420634. https://doi.org/10.3389/fvets.2024.1420634 (2024).

30. Kanehisa, M., Furumichi, M., Sato, Y., Matsuura, Y. & Ishiguro-Watanabe, M. KEGG: biological systems database as a model of the real world. *Nucleic Acids Res.* **53** (D1). https://doi.org/10.1093/nar/gkae909 (2025). D672-D677.
31. Institute, S. P. S. S. SPSS user's guide: Statistics. Version 23.0. SPSS Institute, Chicago, Illinois, USA. (2015).
32. Li, Y., Wang, Y., Shen, X. & Liu, F. The combinations of sulfur and molybdenum fertilizations improved antioxidant capacity of grazing Guizhou semi-fine wool sheep under copper and cadmium stress. *Ecotoxicol. Environ. Saf.* **222**, 112520. https://doi.org/10.1016/j.ecoenv.2021.112520 (2021).
33. Liang, H. et al. Establishment of a mouse model of ovarian oxidative stress induced by hydrogen peroxide. *Front. Vet. Sci.* 111484388. https://doi.org/10.3389/fvets.2024.1484388 (2024).
34. Tan, B. L., Norhaizan, M. E. & Liew, W. P. Nutrients and Oxidative Stress: Friend or Foe? Oxid Med Cell Longev. 9719584. (2018). https://doi.org/10.1155/2018/9719584 (2018).
35. Chen, S. et al. Impact of fermented feed of soybean hulls and rapeseed cake on immunity, antioxidant capacity, and gut microbiota in Chahua chicken. *Poult. Sci.* **103** (3), 103451. https://doi.org/10.1016/j.psj.2024.103451 (2024).
36. Omachi, D. O., Aryee, A. N. A. & Onuh, J. O. Functional lipids and cardiovascular disease reduction: A concise review. *Nutrients* **16** (15), 2453. https://doi.org/10.3390/nu16152453 (2024).
37. Han, H. L. et al. Effects of taurine on growth performance, antioxidant capacity, and lipid metabolism in broiler chickens. *Poult. Sci.* **99** (11), 5707–5717. https://doi.org/10.1016/j.psj.2020.07.020 (2020).
38. Fernandes, A. M. et al. Sustainable fish meal-free diets for Gilthead sea Bream (sparus aurata): integrated biomarker response to assess the effects on growth performance, lipid metabolism, antioxidant defense and immunological status. *Anim. (Basel)*. **14** (15), 2166. https://doi.org/10.3390/ani14152166 (2024).
39. Zhao, X. et al. Effects of compatibility of Clostridium butyricum and Bacillus subtilis on growth performance, lipid metabolism, antioxidant status and cecal microflora of broilers during the starter phase. *Anim. Biosci.* **37** (11), 1933–1944. https://doi.org/10.5713/ab.24.0132 (2024).
40. Zhang, Y. et al. Glycerophospholipid metabolism changes association with Ozone exposure. *J. Hazard. Mater.* **475**, 134870. https://doi.org/10.1016/j.jhazmat.2024.134870 (2024).
41. Jia, L. et al. Phosphatidylcholine ameliorates lipid accumulation and liver injury in high-fat diet mice by modulating bile acid metabolism and gut microbiota. *Inter J. Food Sci. Nutr.* **4**, 1–14. https://doi.org/10.1080/09637486.2024.2437469 (2024).
42. Tian, J. et al. DHA-enriched phosphatidylserine alleviates bisphenol A-induced liver injury through regulating glycerophospholipid metabolism and the SIRT1-AMPK pathway. *Heliyon* **10** (14), e34835. https://doi.org/10.1016/j.heliyon.2024.e34835 (2024).
43. Su, D. et al. Study on the new anti-atherosclerosis activity of different herba patriniae through down-regulating lysophosphatidylcholine of the glycerophospholipid metabolism pathway. *Phytomedicine* **94**, 153833. https://doi.org/10.1016/j.phymed.2021.153833 (2022).
44. Nayyar, D. et al. Effect of a high Linoleic acid diet on pregnant women and their offspring. *Nutrients* **16** (17), 3019. https://doi.org/10.3390/nu16173019 (2024).
45. Liu, J. et al. Linoleic acid promotes mitochondrial biogenesis and alleviates acute lung injury. *Clin. Respir J.* **18** (9), e70004. https://doi.org/10.1111/crj.70004 (2024).
46. Qiu, J. et al. Linoleic acid exhibits anti-proliferative and anti-invasive activities in endometrial cancer cells and a Transgenic model of endometrial cancer. *Cancer Biol. Ther.* **25** (1), 2325130. https://doi.org/10.1080/15384047.2024.2325130 (2024).
47. Zhang, Y. et al. Arachidonic acid metabolism in health and disease. MedComm 4(5), e363. (2020). https://doi.org/10.1002/mco2.363 (2023).
48. Qin, X. et al. Rosiglitazone attenuates acute kidney injury from hepatic ischemia-reperfusion in mice by inhibiting arachidonic acid metabolism through the PPAR-γ/NF-κB pathway. *Inflamm. Res.* **73** (10), 1765–1780. https://doi.org/10.1007/s00011-024-01929-x (2024).
49. Wang, H. et al. Untargeted metabolomics revealed the mechanism of aucubin on glucocorticoid-induced osteoporosis in mice through modulating arachidonic acid metabolism. *J. Pharm. Biomed. Anal.* **248**, 116273. https://doi.org/10.1016/j.jpba.2024.116273 (2024).
50. Ma, Y. et al. Arachidonic acid enhances hepatocyte bile acid uptake and alleviates cholestatic liver disease by upregulating OATP1 expression. *Food Funct.* **15** (19), 9916–9927. https://doi.org/10.1039/d4fo02158d (2024).
51. Jacobs, R. L., van der Veen, J. N. & Vance, D. E. Finding the balance: the role of S-adenosylmethionine and phosphatidylcholine metabolism in development of nonalcoholic fatty liver disease. *Hepatology* **58** (4), 1207–1209. https://doi.org/10.1002/hep.26499 (2013).

Author contributions

Y.F.L.: Conceptualization, Methodology, Software, Formal analysis, Resources, Writing-reviewing & editing, Supervision, Project administration, Funding acquisition, Writing-original draft, Writing-reviewing & editing. Y.Y. W.: Conceptualization, Software, Investigation, Resources, Data curation, Writing-original draft, Writing-reviewing & editing. Z.H. M.: Methodology, Software, Formal analysis, Investigation, Data curation.

Funding

This research was supported by Doctoral Research Start-Up Fund of Liaocheng University (2024318052415).

Declarations

Competing interests

The authors declare no competing interests.

Additional information

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-025-01743-3.

Correspondence and requests for materials should be addressed to Y.L.