# A Serum Metabolic Profiling Analysis During the Formation of Fatty Liver in Landes Geese via Gc-tof/ms

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**Research**

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# Abstract

**Background:**During the process of fatty liver production by overfeeding, the levels of endogenous metabolites in the serumof geese would change dramatically. This study investigated the effects of overfeeding on serum metabolism of Landes geese and the underlying mechanisms using a metabolomics approach.

**Results:**Sixty Landes geese of the same age were randomly divided into the following 3 groups: D0 group (free from gavage); D7 group (overfeeding for 7 days); D25 group (overfeeding for 25 days). The results showed that overfeeding significantly increased the body weight and the liver weight of geese. Serum enzymatic activities and serum lipid levels were significantly enhanced following overfeeding.Gas chromatography time-of-flight/massspectrometry (GC-TOF/MS) was employed to explore the serum metabolic patterns, and to identify potential contributors to the formation of fatty liver and the correlatedmetabolic pathways. A large number of endogenous molecules in serum were altered, especially at the late stage of overfeeding (7 days to 25 days). Continuous elevated levels of pyruvic acid, alanine, proline and beta-glycerophosphoric acid and reduced lactic acid level were observed in the serum of overfed geese. Pathway exploration found that the most of significantly different metabolites were involved in various amino acids metabolism, carbohydrate metabolismand lipid metabolism.

**Conclusions:** These findings pinpoint specific metabolite changes and identify potential biomarkers for early diagnosis of fatty liver disease, as well as provide insights into the perturbation of metabolic pathways involved in fatty liver formation.


# Background

The fatty liver in geese, also called foie gras, is looked upon the delicious foods as caviar, black mushroom by the occidental, which has a rich, buttery and delicate flavor [1]. Consumers worldwide enjoy it, and there is a huge international market [2]. In theory, fatty liver is due to the imbalance of synthesis, secretion and deposition of triglycerides (TG) by the liver [3]. The enhanced TG availability could further disrupt serum biochemical parameters. It was reported that patients with suspected liver damage are initially subjected to liver function tests that include the assessment of alanine transaminase (ALT), aspartate transaminase (AST), and glutamyl transpeptidase (GGT) in serum [4]. Different from human fatty liver, geese have a special ability to store fat in the liver, however, geese generally do not suffer liver fibrosis or liver necrosis, and the functional integrity of the hepatocytes is still preserved [5]. In poultry production, this excellent property is used to produce fatty liver through 2–3 weeks overfeeding [3, 6]. Specifically, Landes geese are famous among waterfowl for their excellent fatty liver production, and the average liver weight can reach 700 to 800 g after a period of overfeeding where high amounts of corn are delivered to the birds to induce liver steatosis [7–9]. For geese, miraculously, severe liver steatosis can spontaneously return to a normal liver without causing any pathological damage [5]. Consequently, Landes geese are considered as an ideal model in biomedical research for the fatty livers of humans and animals.

Metabolomics, as an indispensable platform for system biology and precision medicine, aims to investigate relevant mechanisms by analyzing metabolic profiles of cells, tissues, organs, biofluids, or whole organisms [10–12]. As such, metabolomic has been widely implemented in physiology, disease, and toxicology research fields [13]. Some reports have demonstrated that blood metabolites could be used as physiological biomarkers, reflecting metabolic dysfunction, health and performance in vivo [14, 15]. In the process of liver fattening, no doubt, the levels of endogenous metabolites in response to overfeeding would change dramatically. However, previous studies mainly focused on gene level and confirmed the roles of key genes involved in liver steatosis that regulate diverse functions such as cholesterol, glucose, and lipid metabolism [5, 16, 17]. To date, the biochemical changes in serum caused by liver fattening have not been elucidated from the perspective of metabolomics.

In the present study, in order to understand the serum metabolic mechanism of fatty liver formation, we performed the staged observations of fatty liver formation in Landes geese by overfeeding different days. Meanwhile, for the accuracy and thoroughness, gas chromatography-time-of-flight mass spectrometry (GC-TOF/MS) based metabolomics approach was applied to study the global changes in serum metabolite levels of Landes geese during overfeeding, and to identify potential biomarkers and their involved metabolic pathways so as to timely diagnose fatty liver disease.

## Materials And Methods

The experiment was conducted in accordance with the Chinese Guidelines for Animal Welfare and approved by the Institutional Animal Care and Use Committee of Zhejiang Academy of Agricultural Sciences (Hangzhou, China).

# Experimental animals and samples collection

Sixty healthy and of similar weight Landes geese (65-day-old) were selected for the overfeeding experiment, which was carried out at a farm of ChangXing Glory Goose Industry Co., Ltd. (Huzhou, China). All of these geese grown under natural conditions were fed the same diet. The geese were randomly divided into one control group (n = 20) and two experimental groups (n = 20 in each group). The control group (defined: D0 group) was not subjected to gavage, while the geese in two experimental groups were force-fed with a boiled, maize-based diet (5 meals of 1000 g/day per goose) for 7 days (defined: D7 group) and 25 days (defined: D25 group), respectively. At the end of the overfeeding trial, the geese underwent a night fasting and only water was provided. The morning after, ten geese from corresponding group were selected randomly and weighed individually. Subsequently, blood samples were collected in 1.5 mL Eppendorf tubes by puncture of the wing vein, and then centrifuged at 3000 × *g* 4 °C for 15 min to separate the serum, which was then stored at -80 °C for later analysis. Immediately after blood sampling, the selected geese were slaughtered according to the Administration of Affairs Concerning Experimental Animals of Zhejiang Academy of Agricultural Science. Complete liver stripped from the carcass was weighed and the corresponding value was recorded.

# Detection of serum parameters

Serum biochemical parameters, including ALT, AST, GGT, TG, total cholesterol (TC), and high-density lipoprotein cholesterol (HDL), were measured by using routine enzymatic assays with commercial kits (Jiancheng Bioengineering Institute of Nanjing, Nanjing, Jiangsu, China) with the use of an Automatic Biochemistry Analyzer (Hitachi, Tokyo, Japan).

# Sample preparation for GC-TOF/MS analysis

100 μL of serum from each sample was mixed with 350 μL of methanol and 20 μL of L-2-chlorophenylalanine (1 mg/mL stock in $dH_2O$) in 1.5 mL EP tube. The mixture was vortexed for 15 s, then centrifuged at 12000 × *g* for 15 min at 4 °C to obtain the supernatant. Following, 0.4 mL of supernatant and 60 μL of methoxy amination hydrochloride (20 mg/mL in pyridine) were transferred into the GC/MS glass vial to obtain the extracts, which were dried in a vacuum concentrator at 80 °C for 30 min. Subsequently, 80 μL of bis (trimethylsilyl) trifluoroacetamide regent (BSTFA, 1% TMCS, v/v) was added to each sample, and all the samples were incubated for 1.5 h at 70 °C. In the process of sample preparation, 10 μL of serum from each sample was pooled as a quality control (QC) sample to evaluate the robustness of the following process. Furthermore, 10 μL standard mixture of fatty acid methyl esters (FAMEs, C8-C16: 1 mg/mL; C18-C24: 0.5 mg/mL in chloroform) was added to the QC sample after cooling it to room temperature. Finally, all samples were thoroughly mixed prior to GC-TOF/MS analysis.

# GC-TOF/MS analysis

GC-TOF/MS analysis was performed using an Agilent 7890 gas chromatograph system coupled with a Pegasus HT time-of-flight mass spectrometer. The system utilized a DB-5MS capillary column coated with 5% diphenyl cross-linked with 95% dimethylpolysiloxane (30 m × 250 μm inner diameter, 0.25 μm film thickness; J&W Scientific, Folsom, CA, USA). The aliquot (1 μL) of the analyte was injected in the splitless mode. Helium was used as the carrier gas. The front inlet purge flow was 3 mL $min^{-1}$, and the gas flow rate through the column was 1 mL $min^{-1}$. The initial temperature was kept at 50 °C for 1 min, then raised to 310 °C at a rate of 20 °C $min^{-1}$, and finally kept at 310 °C for 6 min. The injection, transfer line, and ion source temperatures were 280, 270, and 220 °C, respectively. The energy was − 70 eV in electron impact mode. The mass spectrometry data were acquired in full-scan mode with the m/z range of 30–600 at a rate of 20 spectra per second after a solvent delay of 6.17 min.

# Statistical Analysis

The GC-TOF/MS raw data were first processed by Chroma TOF 4.3X software of LECO Corporation and LECO-Fiehn Rtx5 database for raw peak exaction, data baseline filtering and calibration, peak alignment, deconvolution analysis, peak identification, and peak area integration [18]. The resulting normalized data were analysed by multivariate statistical analysis using SIMCA software (version 14.1, MKS Data Analytics Solutions, Umea, Sweden), including principal component analysis (PCA) and orthogonal projections to latent structure-discriminate analysis (OPLS-DA). In addition, the Kyoto Encyclopedia of Genes and Genomes (KEGG, http://www.genome.jp/kegg/) was used to search for the related KEGG pathway of the metabolites. MetaboAnalyst (http://www.metaboanalyst.ca/), which uses the high-quality

KEGG metabolic pathway database as the backend knowledgebase, was used for pathway analysis and visualization.

The experiment data, including the body weight, the liver weight, and the concentrations of serum biochemical parameters, were analyzed using one-way analysis of variance (ANOVA) in SPSS 22.0 software. The significant differences among groups were declared using Tukey's multiple comparison test. All measures of a statistical significance were found when the probability value was less than 0.5. Results presented in this article are shown as mean ± SEM.

## Results

# Body weight, liver weight, and blood index

After 7 and 25 days of overfeeding, the body weights of overfed geese in the D7 and D25 groups were significantly higher ($P < 0.01$) than that of the D0 group (Fig. 1A). The liver weights were considerably higher ($P < 0.01$) of the overfed geese of the D7 and D25 groups and accounted for 4.57% and 12.56% of the body weight respectively, comparing with 2.40% in the D0 group (Fig. 1BC). The effects of overfeeding on the blood indexes of Landes geese are summarized in the Fig. 2. Relative to the D0 and D7 groups, overfeeding significantly increased ($P < 0.01$) the levels of ALT, AST, TC and HDL in the serum of the D25 group. After 25 days of fattening, the amount of GGT in serum was also increased significantly ($P < 0.05$), while the TG did not change dramatically. In addition, no significant statistically difference was found between the D7 and D0 groups in the measured serum biochemical parameters.

# Metabolite detection and identification

The GC-TOF/MS platform was applied to study the response of serum metabolic profile to overfeeding. The total ion chromatograms of goose serum samples from the D0, D7 and D25 groups are shown in Additional file 1: Figure S1. Each peak corresponds to a compound, and the area under the peak represents the relative abundance of the metabolite. In total, 464 peaks were detected by after referring to the LECO-Fiehn Rtx5 database. Further analysis using Chroma TOF 4.3X software to correct the data for missing values, eliminate noise and compliance with an internal standard, 384 valid peaks were retained finally. Among these peaks, 185 compounds were relatively quantified, 126 were marked "analyte" and 73 were labeled "unknown".

# Metabolic profiles of GC-TOF/MS analysis

The multivariate analysis of the metabolic profile differences among the D0, D7 and D25 groups are displayed in Fig. 3. The PCA score plot showed the distribution of origin data, which indicated the overall changes in metabolic physiology under the effects of overfeeding. The comparison results of the three groups all showed slight separation (Fig. 3A). To better describe the contribution of overfeeding for classification and higher level of group separation, the OPLS-DA model was used to clarify the different metabolic patterns. The clear separation and discrimination were found in the OPLS-DA score plot for

each group comparison (Fig. 3B). Further permutation tests were performed to validate the OPLS-DA model. The results of 200 permutation tests showed that the respective $R^2Y$ and $Q^2$ intercept values were 0.90 and − 0.76 in the model of the D0 and D7 groups; 0.85 and − 0.83 in the model of the D7 and D25 groups; and 0.79 and − 0.93 in the model of the D0 and D25 groups (Fig. 3C). The low values of the $Q^2$ intercept represent that the robustness of the model presents a low risk of overfitting and reliability [19]. The $Q^2$ values are all less than 0 in our tests, thereby indicating that the OPLS-DA model can identify the differences between groups and be utilized in downstream analysis.

# Identification of significantly different metabolites in goose serum samples

Significantly different metabolites were identified to further confirm significant variables in goose serum. The criteria for identifying significantly different metabolites are as follows: the variable importance for the projection (VIP) values > 1.0 in OPLS-DA model and *P* values < 0.05 in Student's t-test. Thirty-four significantly different metabolites and 30 significantly different unidentified peaks (named "analyte" or "unknown") between the D7 and D0 groups, 51 and 37 between the D25 and D7 groups, and 55 and 52 between the D25 and D0 groups are listed in Additional file 2: Table S1. These screened metabolites and unidentified peaks are shown in the volcano plots (Fig. 4). Twenty-nine compounds in the serum were upregulated and 35 compounds were downregulated after 7 days of overfeeding (Fig. 4A). In the late stage of overfeeding (7 days to 25 days), 68 upregulated compounds and 20 downregulated compounds were detected (Fig. 4B). For the whole stage of overfeeding (0 day to 25 days), undoubtedly, the molecular substances in the serum changed dramatically, with 75 upregulated metabolites and 32 downregulated metabolites (Fig. 4C). Subsequently, the distinct characteristics of the significantly different metabolites were displayed in the hierarchical clustering heatmap based on the relative abundance of the identified metabolite (Fig. 5). According to the comparison results of the three groups, it can be observed that overfeeding resulted in a continuous increase effect with the number of days in the pyruvic acid, alanine, proline and beta-glycerophosphoric acid. Two other metabolites, 3-hydroxypropionic acid and canavanine, were found to have elevated concentrations in the early stage (0 day to 7 days) and the whole stage (0 day to 25 days). These elevated metabolites may serve as potential biomarkers for the early diagnosis of fatty liver disease. In addition, 25 significantly different metabolites were increased only in the late stage of overfeeding (7 days to 25 days), which are most belonging to organic acids and derivatives (such as succinic acid, succinic acid, 3-aminoisobutyric acid, etc) or lipids and lipid-like molecules (such as 2-hydroxybutanoic acid, alpha-ketoisocaproic acid, squalene, etc). On the contrary, a few significantly different metabolites in serum were reduced following overfeeding. Interestingly, only lactic acid in the three stages of overfeeding was continuously reduced. Furthermore, there was a significant reduction of 7 compounds (including nicotinamide, 2-ketoadipate, oxamic acid, 2-amino-2-methylpropane-1,3-diol, itaconic acid, pelargonic acid, bis(2-hydroxypropyl)amine) in the early stage of overfeeding (0 day to 7 days), and 7 compounds (including sulfuric acid, 5-aminovaleric acid lactam, carnitine, hydrocinnamic acid, uracil-5-carboxylic acid, mucic acid and gentiobiose) in the late stage of overfeeding (7 days to 25 days).

# Characterization and functional analysis of key metabolic pathways

To further explore the effects of the significantly different metabolites and identify potential metabolic pathways that respond to overfeeding, we imported the significantly different metabolites into KEGG. The results showed that the numbers of disturbed metabolic pathways in the early stage (0 day to 7 days), the late stage (7 days to 25 days), and the whole stage (0 day to 25 days) of overfeeding were 22, 32 and 32, respectively. After screening based on the -ln *P*-value and pathway impact scores, the important metabolic pathways are illustrated in a metabolome view map (Fig. 6). Five metabolic pathways were enriched in the D7 group relative to the D0 group (Fig. 6A). Meanwhile, the D25 group was enriched with 9 and 8 metabolic pathways compared with the D7 group and the D0 group, respectively (Fig. 6BC). These key metabolic pathways were classified as various amino acids metabolism (valine, leucine and isoleucine biosynthesis; alanine, aspartate and glutamate metabolism; glycine, serine and threonine metabolism; arginine and proline metabolism; cysteine and methionine metabolism; taurine and hypotaurine metabolism; glutathione metabolism), carbohydrate metabolism (citrate cycle; pyruvate metabolism; glycolysis or gluconeogenesis; glyoxylate and dicarboxylate metabolism; propanoate metabolism), lipid metabolism (linoleic acid metabolism; glycerolipid metabolism). As shown in Table 1, several significantly different metabolites (including pyruvic acid, succinic acid, glyceric acid, fumaric acid, linoleic acid, proline, L-cysteine, threonine, glutathione, etc) were involved in these metabolic pathways, which could be potentially used as biomarkers to diagnose liver injure.

Table 1
Metabolic pathways identified on the significantly different metabolites

| Metabolic pathway | Significantly different metabolites |
|---|---|
| **D7 group vs. D0 group** | |
| Arginine and proline metabolism | (1.626) Citrulline[a] ↑ |
| | (1.566) Proline ↑ |
| | (3.358) Creatine ↑ |
| Nicotinate and nicotinamide metabolism | (0.229) Nicotinamide ↓ |
| Pyruvate metabolism | (1.849) Pyruvic acid ↑ |
| Glycolysis or Gluconeogenesis | (1.849) Pyruvic acid ↑ |
| Glutathione metabolism | (8.101) Glutathione ↑ |
| **D25 group vs. D7 group** | |
| Valine, leucine and isoleucine biosynthesis | (3.002) Threonine ↑ |
| | (2.414) Pyruvic acid ↑ |
| | (2.639) 2-keto-isovaleric acid ↑ |
| Glycine, serine and threonine metabolism | (1.759) Glyceric acid ↑ |
| | (3.002) Threonine ↑ |
| | (2.864) L-Cysteine ↑ |
| | (2.414) Pyruvic acid ↑ |
| Taurine and hypotaurine metabolism | (2.864) L-Cysteine ↑ |
| | (3.261) Taurine ↑ |
| Citrate cycle (TCA cycle) | (2.144) Succinic acid ↑ |
| | (2.414) Pyruvic acid ↑ |
| | (1.699) Fumaric acid ↑ |
| Alanine, aspartate and glutamate metabolism | (1.699) Fumaric acid ↑ |
| | (2.414) Pyruvic acid ↑ |
| | (2.144) Succinic acid ↑ |
| Cysteine and methionine metabolism | (2.473) Cystine ↑ |

[a] The number in the parentheses represents the value of fold change (FC). ↑ and ↓ indicate that the metabolites were upregulated and downregulated in each pair of comparisons.

| Metabolic pathway | Significantly different metabolites |
|---|---|
| | (2.864) L-Cysteine ↑ |
| | (2.414) Pyruvic acid ↑ |
| Glycerolipid metabolism | (3.065) Glycerol ↑ |
| | (1.759) Glyceric acid ↑ |
| Linoleic acid metabolism | (3.011) Linoleic acid ↑ |
| Glutathione metabolism | (3.01E-07) Glutathione ↓ |
| | (2.864) L-Cysteine ↑ |
| **D25 group vs. D0 group** | |
| Valine, leucine and isoleucine biosynthesis | (2.415) Threonine ↑ |
| | (2.029) Leucine ↑ |
| | (4.462) Pyruvic acid ↑ |
| | (3.539) 2-keto-isovaleric acid ↑ |
| Propanoate metabolism | (2.398) Succinic acid ↑ |
| | (7.697) Hydroxypropionic acid |
| | (6.379) 2-Hydroxybutyric acid ↑ |
| Citrate cycle (TCA cycle) | (2.398) Succinic acid ↑ |
| | (4.462) Pyruvic acid ↑ |
| | (1.955) Fumaric acid ↑ |
| Alanine, aspartate and glutamate metabolism | (1.955) Fumaric acid ↑ |
| | (4.462) Pyruvic acid ↑ |
| | (2.398) Succinic acid ↑ |
| Taurine and hypotaurine metabolism | (2.781) Taurine ↑ |
| Glycerolipid metabolism | (2.601) Glycerol ↑ |
| Glyoxylate and dicarboxylate metabolism | (1.828) Glycolic acid ↑ |
| Pyruvate metabolism | (4.462) Pyruvic acid ↑ |

[a] The number in the parentheses represents the value of fold change (FC). ↑ and ↓ indicate that the metabolites were upregulated and downregulated in each pair of comparisons.

# Discussion

What makes geese special is their ability to store energy through overfeeding in the liver to form fatty liver served as a delicious food [20, 21]. In this study, we built a good model of goose fatty liver, with the body weight and liver weight significantly increased after overfeeding for 7 and 25 days. In particular, the liver weight of geese overfed for 25 days increased by approximately 800 g, accounting for about 12% of the body weight. This is in accord with previous results which showed the liver weight increased up to 10-fold after two weeks of overfeeding and accounted for up to 10% of the body weight [22]. Indeed, these results all confirmed the excellent characteristic of fatty liver production in Landes geese.

In clinical livers with disease such as liver cirrhosis and liver steatosis, the detection of serum enzymatic activities is the most direct method to assess liver damage [23, 24]. Serum enzymes mainly come from the liver as the liver steatosis can lead to the hepatocellular inflammation and enzyme synthesis intensification, which will promote the increase of serum enzyme concentration [2]. In the present study, overfeeding for 25 days increased the amounts of serum ALT, AST and GGT, indicating abnormal in liver function of the overfed geese. Similarly, data from published article showed that long-term overfeeding induced liver cell inflammation, accompanied with higher serum enzyme activities [2, 25]. On the other hand, the formation of fatty liver is essentially a disorder of lipid synthesis and secretion, resulting in the accumulation of serum lipid [21]. Our results showed overfeeding for 25 days caused the significant higher levels of TC and HDL, and a numerical increased TG concentration. These variations in serum lipidemic parameters are consistent with previous studies reporting that overfeeding can induce elevated concentrations of serum lipids [21, 26]. Additionally, we noticed that overfeeding for 7 days did not cause significant variation in serum biological parameters, suggesting that short-term fattening may change the apparent performance, while the body's metabolism is still in relatively normal operation.

Different aspects of the development of liver steatosis in Landes geese have been studied under experimental conditions [5, 9, 27, 28]. So far, however, the serum metabolic mechanism in this process has not been clarified. Therefore, metabolic profiling with the aid of GC-TOF/MS combined with multivariate statistical analysis was implemented in our study to explore the serum metabolic patterns, and to identify potential contributors to the formation of fatty liver and the correlated metabolic pathways. In the early stage of overfeeding (0 day to 7 day), 34 differentially expressed endogenous serum metabolites were identified, which were enriched in 22 pathways including arginine and proline metabolism, nicotinate and nicotinamide metabolism, pyruvate metabolism, glycolysis or gluconeogenesis, and glutathione metabolism. In the late stage of overfeeding (7 days to 25 days), the levels of 51 metabolites were significantly changed, which were involved in valine, leucine and isoleucine biosynthesis, glycine, serine and threonine metabolism, taurine and hypotaurine metabolism, citrate cycle (TCA cycle), alanine, aspartate and glutamate metabolism, glycerolipid metabolism, glutathione metabolism, etc. During the whole period of overfeeding, the most differential metabolites were observed, up to 55. The distinct reprogrammed metabolism was mainly involved valine, leucine and isoleucine biosynthesis, propanoate metabolism, TCA cycle, alanine, aspartate and glutamate metabolism, taurine and hypotaurine metabolism, glycerolipid metabolism, glyoxylate and dicarboxylate metabolism, as well as pyruvate metabolism. Just as Ma *et al*. [29] said in the report, the metabolic pathways identified through the significantly different metabolites represent the typical characteristics response of living

systems to pathophysiological stimuli or genetic modification [30]. Comprehensive consideration of our research results, metabolites changed at different stages, such as pyruvic acid, alanine, proline, beta-glycerophosphoric acid, 3-hydroxypropionic acid, canavanine and lactic acid, were selected as potential biomarkers for the early diagnosis of fatty liver disease. In addition, it is noteworthy that several metabolic pathways occur repeatedly at different stages of overfeeding, such as TCA cycle in both the late and the whole stages of overfeeding. The disturbed metabolic pathways identified in the current are consistent with classic metabolism and represent the typical features of the dietary or medical intervention on organisms [31, 32].

In order to systematically demonstrate the metabolic response to overfeeding, the significantly different metabolites combined with corresponding metabolic pathways are shown Table 1. The metabolite with the largest difference between the D0 group and the D7 group was glutathione (8.101 fold higher in the D7 group than that in the D0 group), which is an amino acid and a tripeptide, as well as a well-known antioxidative factor [29]. This finding is in line with a previous study in human, in which γ-glutamyl dipeptide could serve as biomarkers for discrimination among different forms of liver disease, and had a positive correlation with glutathione, providing specific information for different liver diseases [4]. The elevated concentrations of citrulline, proline and creatine, which are products of arginine and proline metabolism, implying the increase of amino acid utilization. Relevant metabolic articles indicated that disturbed metabolism of arginine and proline might play an important role in the obesity progression [33, 34], in complete agreement with the increase in body weight and liver weight of Landes geese.

From the dynamic perspective, the changes of serum metabolism in the late stage of overfeeding were more similar to that in the whole period of overfeeding. The same metabolic pathways screened for these two periods are as follows: valine, leucine and isoleucine biosynthesis; taurine and hypotaurine metabolism; TCA cycle; alanine, aspartate and glutamate metabolism; glycerolipid metabolism. The significantly different metabolites enriched in these pathways were pyruvic acid, succinic acid, fumaric acid, 2-keto-isovaleric acid, glyceric acid, threonine, leucine, l-cysteine, taurine, glycerol, which were all up-regulated in the serum of overfed geese. Notably, pyruvic acid could be found in three metabolic pathways, and it also appeared as a key product in pyruvate metabolism, which can be converted into carbohydrates via gluconeogenesis, to fatty acids or energy through acetyl-CoA, and to amino acids, and ethanol [35]. The elevated level of pyruvic acid in the serum of overfed geese might be related to the intensification of energy conversion. TCA cycle, glycerolipid metabolism and pyruvate metabolism are essential metabolic pathways involved in energy supply. As the center of three major nutrients metabolism (carbohydrates, lipids, and amino acids), TCA cycle is the conversion site among sugar, lipid and amino acid metabolism, and the main way to obtain energy for the body [36]. The concentrations of pyruvic acid, succinic acid and fumaric acid enriched in TCA cycle were increased, indicating that overfeeding exerted an important influence on energy metabolism of Landes geese. Other representative metabolites, such as 2-keto-isovaleric acid, threonine, l-cysteine, leucine, are crucial components involved in protein synthesis. The enhanced of protein synthesis may be the main reason for the improvement of apparent growth performance of overfed geese.

# Conclusion

In summary, our study constructed an excellent model of fatty liver by overfeeding Landes geese, which was proved by the increase of body weight, liver weight, blood lipid concentration and serum enzymatic activities. Although the exact mechanism of serum metabolism during the formation of fatty liver remains unclear, the crucial metabolites as potential biomarkers for the diagnosis of fatty liver and key metabolic pathways were identified in the present study. The specific effects of these metabolites and the interaction mechanisms of these pathways should be further studied in the future. Nevertheless, our results could at least provide possible directions for future research on fatty liver disease.

# Abbreviations

ALT
alanine transaminase; AST:aspartate transaminase; GC-TOF/MS:gas chromatography time-of-flight/mass spectrometry; GGT:glutamyl transpeptidase; HDL:high-density lipoprotein cholesterol; KEGG:Kyoto Encyclopedia of Genes and Genomes; OPLS-DA:orthogonal projections to latent structure-discriminate analysis; PCA:principal component analysis; QC:quality control; TC:total cholesterol; TG:triglycerides; VIP:variable importance for the projection.

# Declarations


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### Availability of data and materials

All data generated or analyzed during this study are available from the corresponding author on reasonable request.

### Authors' contributions

YX and HY designed the study. WL and XS conducted the animal trial and the laboratory work. YG conducted a literature review and analyzed the data. YG and YX wrote manuscript and approved thefinal version. XZ and LLprovided suggestions on the experimental design andthe manuscript.

### Consent for publication

Not applicable.

### Conflict of interest

The authors declare that there are no conflicts of interest.

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# Figures

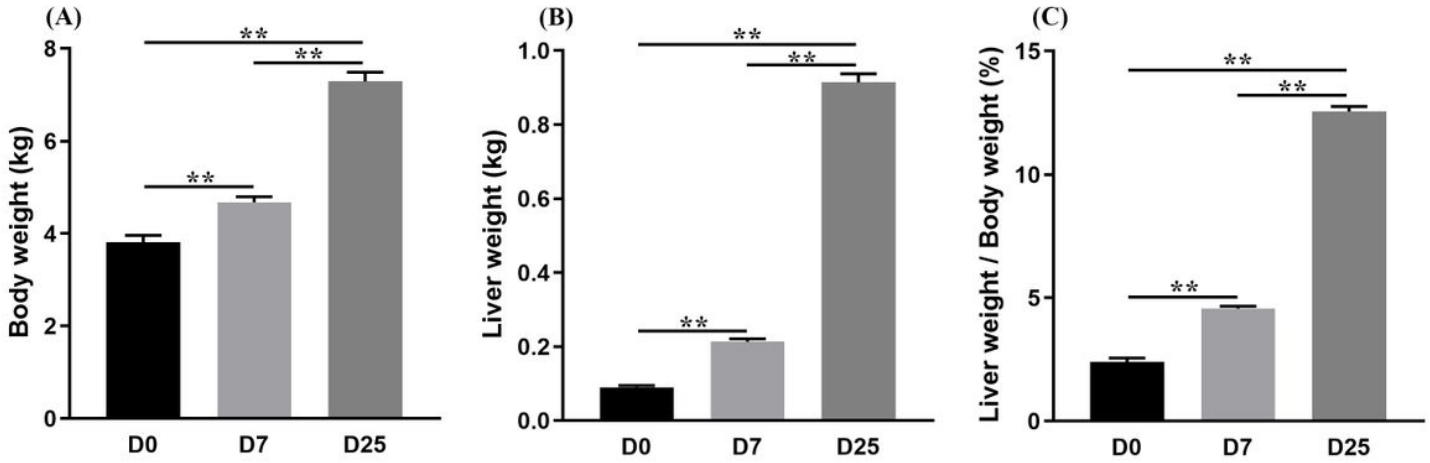
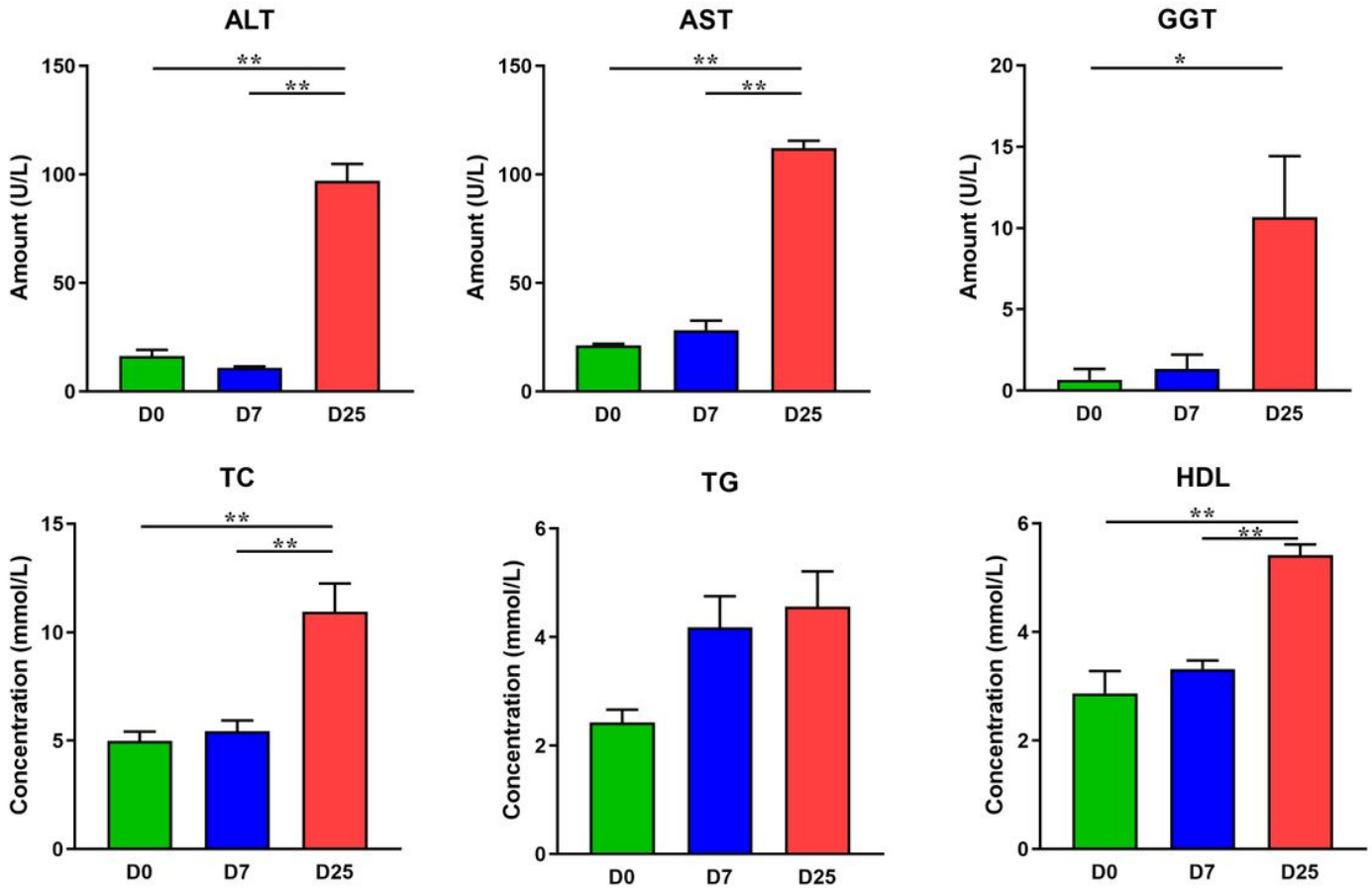



**Figure 1**

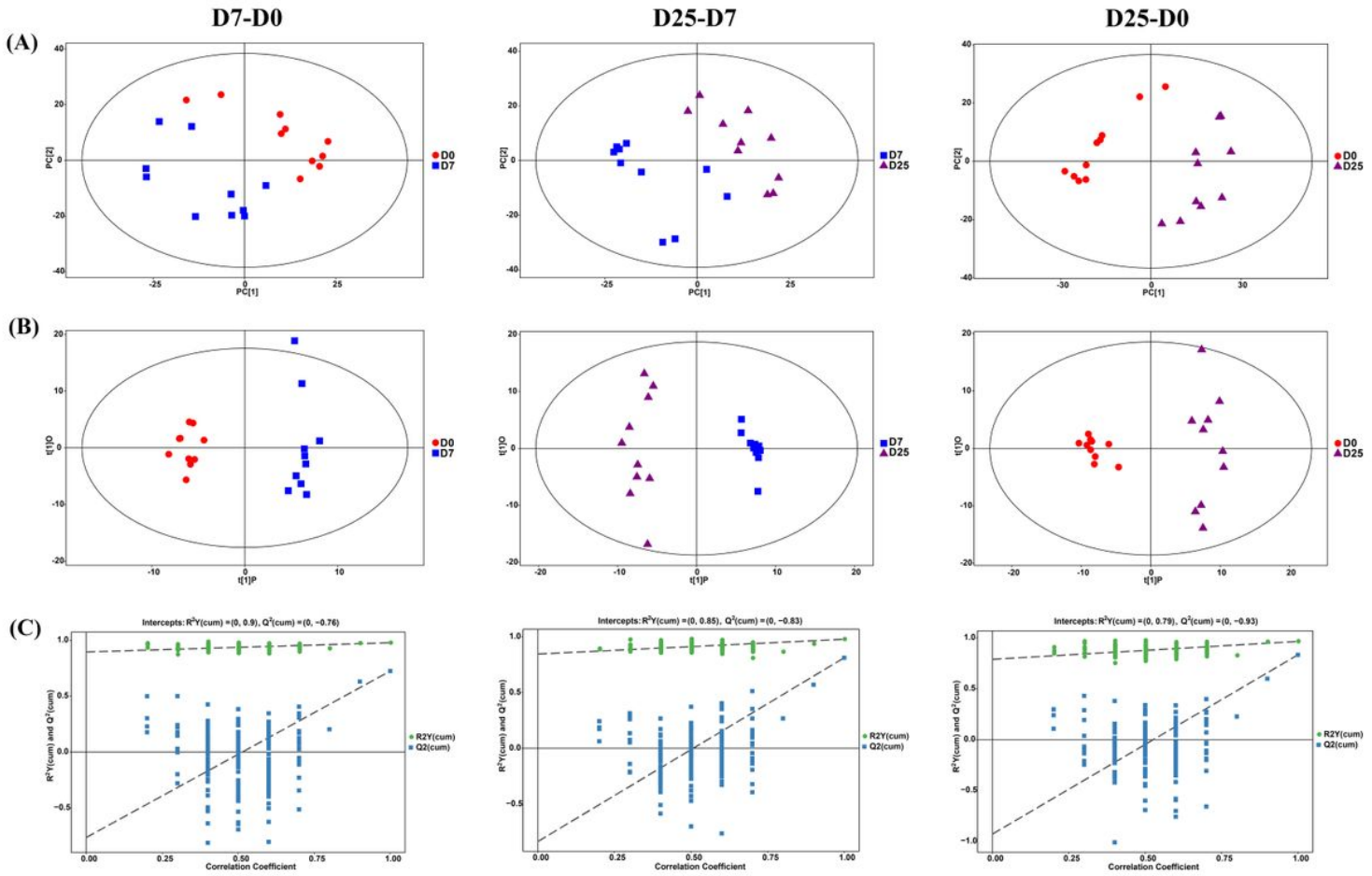
The effects of overfeeding on the body weight (A), liver weight (B), and the ratio of liver weight to body weight (C) of Landes geese. Values are means with their standard errors. Asterisks indicate statistically

significant differences among the groups: *P< 0.05, **P< 0.01. n = 10 per group. D0: overfeeding for 0 day; D7: overfeeding for 7 days; D25: overfeeding for 25 days.



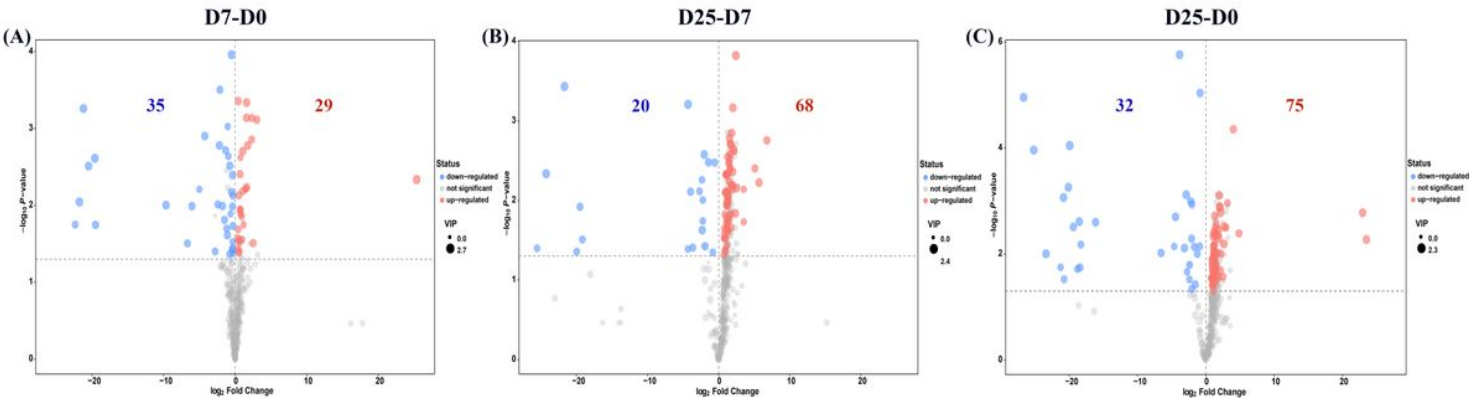

## Figure 2

The effects of overfeeding on theserum biochemical parametersofLandes geese.Values are means with their standard errors. Asterisks indicate statistically significant differences among the groups: *P< 0.05, **P< 0.01. n = 6 per group. ALT: alanine aminotransferase; AST: aspartate aminotransferase; GGT: γ-glutamyl transpeptidase; TC: total cholesterol; TG: triglycerides; HDL: high-density lipoprotein cholesterol. D0: overfeeding for 0 day; D7: overfeeding for 7 days; D25: overfeeding for 25 days.




## Figure 3

The analysis of GC-TOF/MS of geese serum samples. Panel (A) shows the PCA score plots among the D0, D7 and D25 groups. Panel (B) shows the OPLS-DA score plots among the D0, D7 and D25 groups. Panel (C) shows the OPLS-DA corresponding validation plots among the D0, D7 and D25 groups. GC-TOF/MS: gas chromatography-time-of-flight mass spectrometry; PCA: principal component analysis; OPLS-DA: orthogonalpartial least squares discriminant analysis. D0: overfeeding for 0 day; D7: overfeeding for 7 days; D25: overfeeding for 25 days.




## Figure 4

Volcano plots of serum metabolites. Panel (A) shows the D7 group compared to the D0 group. Panel (B) shows the D25 group compared to the D7 group. Panel (C) shows the D25 group compared to the D0 group.The red dots represent metabolites that are up-regulated, the blue dots represent metabolites that are down-regulated, and the gray dots represent metabolites that do not change significantly. The dot sizes indicate the variable importance in the projection (VIP) value. D0: overfeeding for 0 day; D7: overfeeding for 7 days; D25: overfeeding for 25 days.

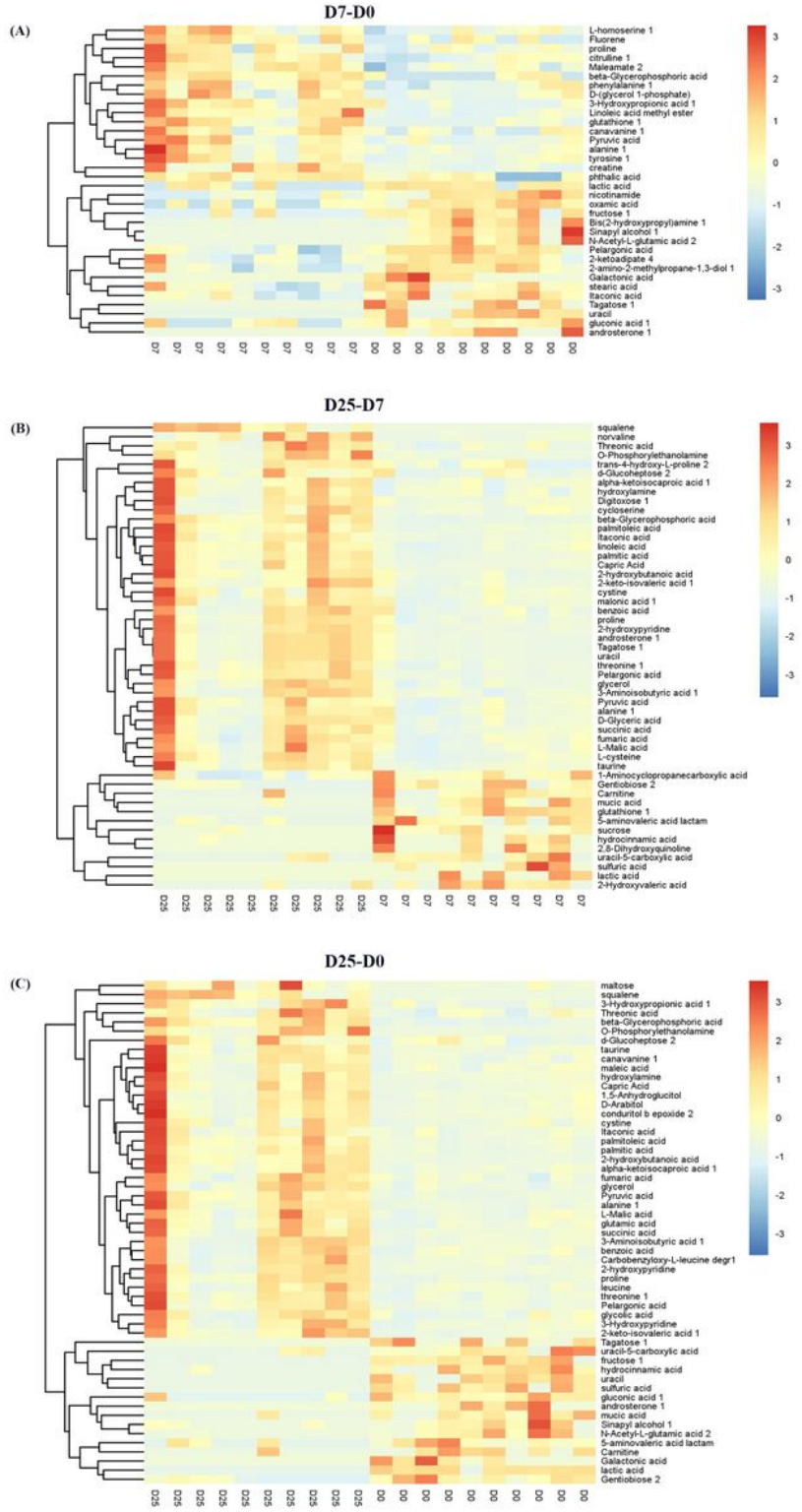



**Figure 5**

Hierarchical clustering analysis for the significantly different metabolites. Panel (A) shows the D7 group compared to the D0 group. Panel (B) shows the D25 group compared to the D7 group. Panel (C) shows the D25 group compared to the D0 group. The relative metabolite levels are expressed based on the color scale. Red represents upregulation, while blue represents downregulation. D0: overfeeding for 0 day; D7: overfeeding for 7 days; D25: overfeeding for 25 days.

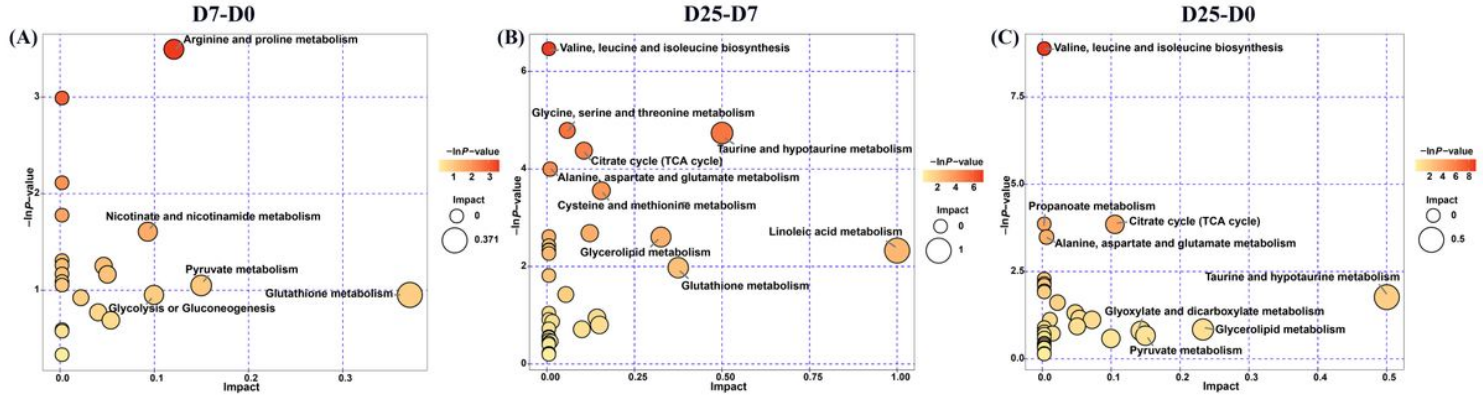



**Figure 6**

Metabolome view map of significant metabolic pathways. Panel (A) shows the D7 group compared to the D0 group. Panel (B) shows the D25 group compared to the D7 group. Panel (C) shows the D25 group compared to the D0 group. Significantly changed pathways are depicted according to enrichment and topology analysis. Large sizes and dark colors represent high pathway impact andmajor pathway enrichment, respectively.D0: overfeeding for 0 day; D7: overfeeding for 7 days; D25: overfeeding for 25 days.

# Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- FigureS1.tif
- TableS1.xlsx