Comparison of performance, fatty acid composition, enzymes and gene expression between overfed Xupu geese with large and small liver

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ABSTRACT

The aim of our study was to compare overfeeding performance, fatty acid composition, enzymes activity and gene expression between overfed Xupu geese with large and small liver. A total of sixty 80 d male Xupu geese were overfed by the same feed intake under the same conditions. The liver weight exhibited normal distribution. Based on the weight of liver, samples were divided into large liver (LL) and small liver (SL) Xupu geese. After overfeeding for 28 d: compared with the SL-Xupu geese, liver weight and liver-to-body weight ratio increased (\( p < .05 \)), while abdominal fat weight and feed-to-liver weight ratio decreased (\( p < .05 \)); the level of palmitic acid and methyltetracontanoate acid decreased (\( p < .05 \)), and the level of oleic acid, linoleic acid, arachidonic acid, and docosahexaenoic acid increased (\( p < .05 \)) in liver of LL-Xupu geese. Compared with the SL-Xupu geese, LL-Xupu geese had a higher plasma concentration of TG (triglyceride) (\( p < .05 \)), and had a lower plasma concentration of VLDL-C (very low density lipoprotein cholesterol) (\( p < .05 \)); and had a higher CHE (cholinesterase), LPS (lipase), LPL (lipoproteinlipase) activities (\( p < .05 \)) in the liver. The mRNA expression of FABP4 (adipocyte fatty acid binding protein), SCD (stearoyl-CoA desaturase), FADS (fatty acid dehydrogenase), ELOVL1 (elongase of long-chain fatty acid 1), ACAT2 (acyl-Co A: cholesterol acyltransferase 2), and FASN (fatty acid synthase) (\( p < .05 \)) were significantly upregulated in LL-Xupu geese than in SL-Xupu geese. The results showed that the level of unsaturated fatty acid in liver of LL-Xupu geese was higher than that of SL-Xupu geese, which may be closely related to enzymes and genes expression related to lipid metabolism.

HIGHLIGHTS

- The content of unsaturated fatty acids in liver of LL-Xupu geese were significantly higher than in SL-Xupu geese.
- The mRNA expression of FABP4, SCD, FADS, ELOVL1, ACAT2, and FASN were significantly upregulated in LL-Xupu geese than in SL-Xupu geese.
- The level of unsaturated fatty acid in liver of LL-Xupu geese was higher than that of SL-Xupu geese, which may be closely related to enzymes and genes expression related to lipid metabolism.

Introduction

In recent years, the protection and exploitation of indigenous Chinese geese have drawn increasing attention and concern (Lin et al. 2019; Ma et al. 2020). The Xupu geese (Anser cygnoides domesticus) is a dual-purpose indigenous geese breed in China, which produces both fatty liver (Foie gras) and meat. ‘Foie gras’, known as delicate texture, delicious taste, mellow fragrance, and rich nutrition, is considered as one of the most delicious foods in the world (Liu et al. 2016).

Xupu geese, a local breed in the List of National Livestock and Poultry Genetic Resources Protection of China (Dai et al. 2016), mainly thrive in the Western Hunan province of China. A study on the genetic background of this breed indicated that this domestic geese originated from the Anser cygnoides (Liu et al. 2016). Compared with other domestic geese breeds, Xupu geese has a high capacity of fat accumulation in liver, which make a 5 to 10-fold increase in liver size after short-term overfeeding, and resulting fatty liver is usually composed of above 50% unsaturated fatty acids.
acid (UFA) (Hermier et al. 1994, 1999). However, the breed shows variable liver weight after overfeeding due to lack of scientific breeding programs, which was assumed to impede the production efficiency of commercial ‘Foie gras’, and had an impact on the utilisation and conservation of genetic resources of Xupu geese (Zhu et al. 2011).

To our knowledge, this is the first study to investigated liver fat deposition, plasma biochemistry indices, histological analysis, fatty acid composition, enzymes and genes expression related to lipid metabolism between LL and SL-Xupu geese. These data not only laid the foundation for the study of liver fat deposition mechanism, but also provided a theoretical reference for the development of ‘Foie gras’ food.

Material and methods

Ethical statement

The experiment was approved by the Institutional Animal Care and Use Committee of the Hunan Agricultural University (Hunan, China). The methods were performed in accordance with the approved guidelines and regulations of the regional Animal Ethics Committee (Changsha, China).

Experimental design, diets, and management

A total of sixty 80 d healthy males Xupu geese from the same population and averaging body weight (4,947.00 ± 267.54 g) were housed in the same room at the Hunan Hongyu Xupu geese Development Co., Ltd. (Hunan, China). Each goose was ringed and marked with a plastic wing-tag for individual identification. All geese were pre-overfed for a week, during which the food intake was increased progressively to enlarge the volume of the digestive tract and to initiate metabolic adaptation to overfeeding. At the end of the pre-overfeeding period, all geese were forcefed with a carbohydrate diet consisted of 98% boiled whole maize, 1.0% waterfowl fat, 0.5% salt and 0.5% vitamin (3,370 kcal/kg, 90 g of protein/kg, and 4.5 g of fat/kg). The geese were overfed 3 times each day at an interval of eight hours with 550 g high carbohydrate diet in the first week of the overfeeding period. The next week, the geese were then given 4 meals of 1,200 g/d, following by the overfeeding of 5 meals (1,600 g/d) for the last two weeks.

This overfeeding experiment was conducted from July to August in 2018 at the Hunan Fu’e Development Co., Ltd. (Hunan, China). Geese were housed in an environmentally controlled room. During the experiment, the temperature and relative humidity in the room were 28 ± 1.06°C and 60.86%±0.88%, respectively. All geese were reared on the ground (4 m × 6 m). Each goose was labelled with a ring on its right foot. In our study, we used an overfeeding machine that can adjust the feed weight, and operated by the same professional staff each time. During the overfeeding period, all geese had free access to water at all times.

Sample collection

After 28 days of overfeeding, all geese were provided with water but deprived of food overnight for 12 h. On the following morning, they were weighed and then blood samples were withdrawn by puncture of the occipital venous sinus and placed at room temperature for 1 h, a plasma sample was obtained by centrifugation at 3,000 × g for 20 min at 4°C. After blood sampling, the geese were killed by exsanguination and whole liver was removed quickly and weighed it.

Samples with body weight gain rate between 75 and 90% for data analysis. The liver weight of 42 samples were in accordance with normal distribution (Figure 1). On the basis of the wide liver weight distribution, eight geese with the highest and lowest liver
weight were selected and designated as large liver Xupu geese (LL-Xupu geese) and small liver Xupu geese (SL-Xupu geese) groups, respectively. Liver samples were immediately taken from the ventromedial portion of the main lobe (right lobe) and frozen in liquid nitrogen and stored at −80°C till analysis of enzymes activity and mRNA level.

**Histological evaluation**

Small fresh tissue pieces (5 mm × 5 mm × 5 mm) of liver from eight LL-Xupu geese and eight SL-Xupu geese were removed and immersed in 10% neutral buffered formalin. The fixed tissues were processed and embedded in paraffin by routine histologic methods. 3 to 4 μm sections were stained with haematoxylin and eosin, and tissue was evaluated using a light microscope. The liver samples were processed for histological examination according to Bancroft and Gamble (2008).

**Fatty acid composition**

The fatty acid composition of liver was determined according to the previously described method (Li et al. 2015). Briefly, total lipids were extracted from the liver tissue (lyophilized sample) using petroleum ether/anhydrous diethyl ether (1:1, vol/vol). Methyl esters of the lipids were prepared using saponification with a solution of KOH: methanol (4 mol:1 L), and the organic layer was aspirated for the analysis of fatty acids with an Agilent 7890 N gas chromatography equipped with a flame ionisation detector (Agilent Technologies) and a CP-Sil 88 fused silica open tube capillary column (100 m × 0.25 nm; Chrompack). Sample (1 μL) was injected into the gas chromatography port by the auto sampler. The gas chromatograph temperature program was as follows: initial temperature of 140°C for 5 min, an increase of 3°C/min to 220°C, 1 min at 220°C, and then holding at 220°C for 40 min. The injector and detector temperatures were maintained at 240°C and 260°C, respectively. Hydrogen was used as the carrier gas at a flow rate of 40 mL/min. Individual fatty acid peaks were identified by comparing their retention times with those of the standards (Cat#: 18919-1AMP; Sigma Chemicals, St. Louis, MO, USA). The concentration of individual fatty acids was quantified according the peak area, and expressed as a percentage of total fatty acids.

**Plasma biochemistry and lipid metabolism-related enzymes activities**

Concentrations of triglyceride (TG), cholesterol (TC), low density lipoprotein cholesterol (LDL-C), very low-density lipoprotein cholesterol (VLDL-C), and high density lipoprotein cholesterol (HDL-C) in plasma were measured by using an Mindray automatic analyser (BS-300; Shenzhen Mindray Bio-Medical Electronics Co., Ltd, Shenzhen, Guangdong, China) according to the commercial kits (Shenzhen Mindray Bio-Medical Electronics Co., Ltd). Activities of cholinesterase (CHE, A054-1), lipase (LPS, A023-1), lipoproteinlipase (LPL, A067), hepaticlipase (HL, A067), nonesterified free fatty acids (NEFA, A042) were determined using corresponding diagnostic kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) with Microplate reader (Multiskan GO; Thermo Fisher Scientific, Waltham, CT, US) according to the instructions of the manufacturer.

**Liver lipid metabolism-related enzymes activities**

Approximately 0.5 g of liver sample was used to prepare the tissue homogenate. Tissues were diluted in 1:9 (W/V) with ice-cold 154 mmol/L sodium chloride solution, and homogenised using an Ultra-Turrax homogeniser (T10BS25, IKA, Baden-Wurttemberg, Germany), then centrifuged at 3,500 × g at 4°C for 10 min. The supernatant was used for analyses the activities of CHE, LPS, LPL, HL, and content of NEFA. Protein concentration in the supernatant of the liver homogenate was measured by using a protein assay kit (A045-2; Nanjing Jiancheng Institute of Bioengineering).

**Total RNA extraction, reverse transcription, and quantitative real-time PCR**

Total RNA from liver tissues was isolated using TaKaRa MinBEST Universal RNA Extraction Kit (Takara, Osaka, Japan) according to the manufacturer’s protocol. The concentration and purity of RNA were determined using a Nano-Drop 2000 Spectrophotometer (Thermo Scientific, Hudson, NH, USA), and the integrity of RNA was analysed by 1% agarose gel electrophoresis. Only RNA specimens with an A260/A280 ratio of 1.8–2.0 and an A260/A230 ratio ≥ 2.0 were used for subsequent analysis. In order to avoid genomic DNA contamination, the total RNA was treated with DNase I using the PrimeScript RT reagent Kit with gDNA Eraser kit (Takara, Osaka, Japan). DNA-free RNA was reverse transcribed into cDNA, and cDNA was then diluted.
Table 1. The primers used for gene cloning, quantitative real-time PCR.

| Gene name | Accession number | Forward sequence (5’ to 3’) | Reverse sequence (5’ to 3’) |
|-----------|------------------|-----------------------------|-----------------------------|
| FABP4     | H083818.1        | CAAGCCAAATTGACTATCA         | ATCAAAACTTCATCCAACT        |
| SCD       | H917924.1        | CTGGGTGGTGTGTGTGCT          | GGGTCTCCCGGGTGTTGAT        |
| FADS      | XM01377620.1     | ATCTTTACAGTCCTTAGGCA        | ACAGAGTGTTTTTTCCCA         |
| ELOVL1    | XM01377961.1     | TTTGGAACAGGGGGA             | GGAGATGTGGAGCAAGAGG        |
| ACAT2     | XM01386797.1     | GGAACCGGAGCGGTAAC           | GAGCCAAAGGCAATAA           |
| LPL       | XM01388252.1     | TACTGGAAGACCGGTTGC          | ACCTTCATCTAAGTGCCATTAC     |
| FASN      | XM01397391.1     | AATCCAGAAGGCGCA             | TCCAGCAATGGCGTTAAG         |
| GAPDH     | XM027449739.1    | GCCGAGAATTATTACCCA          | CAGGTCAGGTCAGCAGA          |
| β-actin   | M26111.1         | CGAGGGTTCAGGGGTTCA          | GTCTGATCTCCTGGCTTG         |

qPCR: quantitative real-time PCR; FABP4: fatty acid binding protein gene; SCD: steroyl-CoA desaturase gene; FADS: fatty acid dehydrogenase gene; ELOVL1: elongase of long-chain fatty acid 1 gene; ACAT2: acyl-Co A: cholesterol acyltransferase 2 gene; LPL: lipoprotein lipase gene; FASN: fatty acid synthase gene; GAPDH: glyceraldehyde-3-phosphate dehydrogenase gene.

1:10 with nuclease-free water before being used for Quantitative real-time PCR. The primer pairs for the amplification of adipocyte fatty acid binding protein gene (FABP4), steroyl-CoA desaturase gene (SCD), fatty acid dehydrogenase gene (FADS), elongase of long-chain fatty acid 1 gene (ELOVL1), acyl-Co A: cholesterol acyltransferase 2 gene (ACAT2), lipoprotein lipase gene (LPL), fatty acid synthase gene (FASN), glyceraldehyde-3-phosphate dehydrogenase gene (GAPDH), and β-actin gene were designed from GenBank sequences using Primer Premier 5.0 and obtained from Shanghai Sangon Biotech Co., Ltd. (Shanghai, China), as shown in Table 1.

Quantitative real-time PCR (qPCR) was performed using SYBR Green Master Mix (Vazyme Biotech, Nanjing, China) in CFX96 TouchTM Real-Time PCR Detection System (Bio-Rad Laboratories, USA). The PCR systems consisted of 2 μL of diluted cDNA template (1:9), 12.5 μL of SYBR® Premix Ex Taq™ II, 1 μL PCR Forward Primer (10 μmol/L), 1 μL PCR Reverse Primer (10 μmol/L) and 8.5 μL of sterilised distilled water. The PCR programs were as follows: 95°C for 30 s, followed by 35 cycles of denaturation at 95°C for 5 s and 60°C for 30 s. Dissociation curves of the products were generated by increasing the temperature of samples incrementally from 55 to 95°C as the final step of the PCR. GAPDH and β-actin genes were used as the dual internal standard for normalising transcript abundance of mRNA expression. The relative expression levels of target genes were calculated by the 2^ ΔΔCt method as described by Livak and Schmittgen (2001).

Statistical analysis

Univariate analysis (SPSS 21.0) was used to analyse the liver weight to determine its normal distribution, and data from the two tails were compared by Student’s t test (SAS 9.3). Variability of all the data was expressed as standard error of the mean (SEM). Differences between mean values were compared using Student’s t test, and considered significant at p < .05.

Results

Overfeeding performance

The results for overfeeding performance of LL-Xupu geese and SL-Xupu geese are presented in Table 2. There were no significant differences in the initial weight, final weight, and body weight gain rate between LL and SL-Xupu geese. Compared with the SL-Xupu geese, liver weight and liver-to-body weight ratio increased (p < .05) and abdominal fat weight and feed-to-liver weight ratio decreased (p < .05) in the LL-Xupu geese.

Histological evaluation

Histological evaluation of LL and SL-Xupu geese is summarised in Figure 2. Some morphological differences were observed between the livers sections obtained from LL and SL-Xupu geese markedly displayed differences. In Figure 2(A), the cell boundaries of hepatocytes were visible, while the nuclei of hepatocytes were centrally located in SL-Xupu geese. However, the cell boundaries were blurry in LL-Xupu geese, as shown in Figure 2(B). In contrast, we clearly observed many lipid droplets, and the nuclei were compressed to the edges of the cells in LL-Xupu geese.

Fatty acid composition

The fatty acid composition in LL and SL-Xupu geese are shown in Table 3. The major fatty acids were C16:0, C18:0, C16:1, C18:1n-9, and C18:2n-6. Oleic acid (C18:1n-9) was the most abundant fatty acid, accounting for 58.15% and 54.68% of total fatty acid in liver of LL and SL-Xupu geese, respectively. Compared to the liver of SL-Xupu geese, the level of saturated fatty acids (SFA), including palmitic acid (C16:0) and methyltetrasanoate acid (C24:0) decreased (p < .05), and the level of...
polyunsaturated fatty acids (PUFAs), including oleic acid (C18:1n-9), linoleic acid (C18:2n-6), arachidonic acid (C20:4n-6), and docosahexaenoic acid (C22:6n-3) increased ($p < .05$) in liver of LL-Xupu geese. However, no significant differences were found for myristic acid (C14:0), stearic acid (C18:0), methyleicosanoic acid (C20:0), palmitoleic acid (C16:1), elaidic acid (C18:1t9), eicosenoic acid (C20:1) between SL and LL-Xupu geese.

**Blood chemistry**

As is shown in Table 4, the results of blood chemistry of LL and SL-Xupu geese revealed that there were no significant differences in TC, LDL-C, and HDL-C between LL and SL-Xupu geese. The concentration of TG was significantly higher ($p < .05$), while the concentration of VLDL-C was significantly lower ($p < .05$) in the LL-Xupu geese than that of SL-Xupu geese.
Lipid metabolism-related enzymes activities

The results of lipid metabolism-related enzyme activities in the plasma and liver of LL-Xupu geese and SL-Xupu geese are presented in Table 5. Among the lipid metabolism-related enzymes indexes, a significant enhancement (p < .05) of the CHE, LPS, and LPL were observed in liver of LL-Xupu geese compared to the SL-Xupu geese.

Gene expression

The lipid metabolism-related gene mRNA expression in livers between LL and SL-Xupu geese are shown in Figure 3. The mRNA expression of FABP4, SCF, FADS, ELOVL1, ACAT2, and FASN in liver of LL-Xupu geese were significantly upregulated (p < .05).

Discussion

The liver weight positively influences liver quality, including favour, juiciness and tenderness in different breeds. The distribution of the liver weight within overfed native geese showed large variation, which may be mostly regulated from the genetic level. Xupu geese is generally classified as large geese in size and exhibited a better capacity of fat deposit in liver than other Chinese native geese breeds. Previous research showed that the average liver weight of Xupu geese was 600.3 g after 28 days of overfeeding, and the maximum and minimum liver weight was 929 g and 187 g, respectively (Mao 1984). Fernandez et al. (2019) compared characteristics in livers obtained by overfeeding or by spontaneous fattening, and found that the average liver weight of geese were 1102 g and 445 g in overfeeding group and spontaneous fattening group. In our study, all of the geese were sampled from a natural population. The results showed that the average liver weight of LL-Xupu geese was 977.17 g, which was more than four time that of SL-Xupu geese, and the average abdominal fat weight of LL-Xupu geese was 487.50 g, which was lighter than that of SL-Xupu geese. The liver weight of overfed Xupu geese were detected in a normal distribution. Accordingly, there was a significant difference in liver size, and liver-to-body weight ratio of LL-Xupu geese was significantly higher than that of SL-Xupu geese, and feed-to-liver weight ratio was significantly lower than that of SL-Xupu geese, while there were no differences in initial weight, final weight, and body weight gain rate between two groups, the differences in liver may be associated with an imbalance between lipid synthesis and secretion. The fact that de novo hepatic lipogenesis from dietary carbohydrates dramatically enhanced in LL-Xupu geese resulted in the accumulation of lipids. The remarkable increase in abdominal fat weight
in SL-Xupu geese suggests that an extrahepatic tissue lipolysis inhibition could contribute to the higher peripheral fattening in SL-Xupu geese and could be a restrictive factor of hepatic steatosis in SL-Xupu geese.

In the present study, geese were overfed in the same type of feed (corn) during the overfeeding period. The differences observed in the saturation level of the fatty acids that form the component chains of TG are most likely due to the significant increase in TG plasma in liver of LL-Xupu geese. TG as the main raw material for the synthesis of fatty acids, and numerous studies found that excessive accumulation of TG is the main characteristic of hepatic steatosis (Guo et al. 2017; Ji et al. 2017). A previous in vitro study also found insulin and glucose increased TG accumulation in geese primary hepatocytes (Han et al. 2009). Different regulation patterns of the enzymes activities related to lipid metabolism in liver steatosis. The experiment showed that there were no significant differences except for the significant increase in the CHE, LPS, and LPL activities in liver of LL-Xupu geese. In addition, the concentration of VLDL-C decreased significantly in the LL-Xupu geese than that of SL-Xupu geese. The amount of synthetic fat is much more than the amount of fat transported, causing a large accumulation of fat in the liver to form fatty liver. Some researchers reported that insulin and glucose affects geese hepatocellular lipid metabolism through the PI3K-Akt-mTOR signalling pathway (Han et al. 2015).

A growing body of researches confirmed that saturated fatty acids (SFA) will be associated with increased risk of cardiovascular disease and incident coronary heart disease (Smith et al. 2020). Many authorities and countries introduce dietary recommendations for the general population to restrict intake of SFA, and increase the intake of UFA. Our study showed that polyunsaturated fatty acids (PUFA) in liver of LL-Xupu geese were significantly higher than in SL-Xupu geese. It is worth noting that the UFA content was more than 50% in both groups. Besides, geese fatty liver may serve as a unique model of fatty liver, as it differs from mammalian non-alcoholic fatty liver disease in which that geese may have a protective mechanism that prevents toxicity associated with severe hepatic steatosis (Geng et al. 2015, 2016; Liu et al. 2016). In agreement with previous studies (West et al. 1992; Jiang et al. 2011; Ghafari et al. 2016), the higher fatty acids in the liver are oleic acid (C18:1n-9), palmitic acid (C16:0), stearic acid (C18:0), and linoleic acid (C18:2n-6) in liver of Xupu geese. Palmitic acid (C16:0), the main lipid produced by de novo lipogenesis, is the starting point for the synthesis of the various fatty acids, including monounsaturated and long chain polyunsaturated fatty acids, that are incorporated in TG, together with the fatty acids ingested directly in the food (Fernandez et al. 2019). In rodents, about 90% of the stearic acid is synthesised from palmitic acid by elongase activity (Moon et al. 2014). Our previous studies also found that oleic acid was the highest fatty acid in the liver of Xupu geese and Landes geese (Liu et al. 2020). In this study, C16:0 and methyltetrascanoate acid in liver of SL-Xupu geese were significant higher than that of LL-Xupu geese. In accordance, the level of SFA in liver of LL-Xupu geese was significant lower than that of SL-Xupu geese. While there were no significant differences in stearic acid between two groups, which maybe closely related to elongating enzyme activity (da Silva-Santi et al. 2016). Another interesting observation from the current study was that the level of PUFA of LL-Xupu geese were significantly higher than that of SL-Xupu geese, while the level of MUFA has no significant difference. PUFA exhibit to better health by reducing inflammatory disorders and metabolic disease-related pathologies (Liao et al. 2014; Reyes-Quiroz et al. 2014; Gu et al. 2015).

During overfeeding, geese develop severe hepatic steatosis, and hepatic de novo lipogenesis is enhanced. Many researches showed that the development of severe steatosis is closely related to the genes involved in lipid synthesis, packaging, secretion, transportation, deposition or metabolism (Zhang et al. 2014; Milano et al. 2015). FADS play an important role in the synthesis of monounsaturated fatty acids (MUFAs) from SFAs (Moltó-Puigmartí et al. 2010). Osman et al. (2016) found that FADS1 and FADS2 as protective components were promoted to meet the instant need for LC-PUFAs (long chain polyunsaturated fatty acids) in geese fatty liver, and proposed this was required for severe hepatic steatosis without liver injury. The results indicated FADS may be a potential therapeutic target as FADS expression and mutations were associated with liver fat. SCD, a FADS member gene, is an endoplasmic reticulum enzyme that catalyses the biosynthesis of MUFAs from SFAs and is the critical gene responsible for the synthesis of TG, phospholipids, and cholesterol esters (Marklund et al. 2018; During et al. 2020). SCD plays an important role in TG accumulation, as overfeeding markedly increased the mRNA expression of SCD in the liver of Sichuan White geese and Landes geese, and the mRNA abundance of SCD in the liver had significant positive correlations with TG content in liver lipids and in the levels of
plasma insulin and VLDL levels in Sichuan white geese (Zhang et al. 2013). The ELOVL1 exhibits fatty acyl-coenzyme A elongase activity specific to long chains (C22 to C24 and C26 saturated and monounsaturated fatty acids) and is important for tissue fatty acid composition. Ofman et al. (2010) demonstrated that knockdown of ELOVL1 lowers C26:0 synthesis and C26:0 levels in X-linked adrenoleukodystrophy (X-ALD) fibroblasts, which provided ELOVL1 inhibition could be used as a therapeutic option for X-ALD. In the present study, compared to SL-Xupu geese, we found that genes implicated in the biosynthesis of UFAs (such as FADS, SCD, and ELOVL1) increased in LL-Xupu geese. And the results were in agreement with the researches of Schackmann et al. (2015). Furthermore, FABP4 plays a key role in fatty acid uptake and intracellular transport. Uysal et al. (2000) reported that knockout of FABP4 in mice reduces plasma triglyceride and cholesterol and improves lipid metabolism. ACAT is the only known enzyme in cells that catalyzes the formation of cholesterol esters from free cholesterol and long-chain fatty acids. It is an extremely important enzyme involved in the absorption, transport, and storage of cholesterol. ACAT2 of hepatocytes is responsible for producing cholesterol esters, synthesising, assembling and secreting VLDL. Brown et al. (2008) found that ACAT2 activity in liver could increase the production of cholesterol esters and the secretion of ApoB and lipoprotein (VLDL, MDL, LDL) in liver. However, we found that ACAT2 gene expression increased in the LL-Xupu geese, which may be the reason of a certain degree of lipolysis. This part of result needs further study. FAS mainly exists in liver, fat and other tissues, and its activity will directly control the synthesis of fatty acids in vivo, thus playing an important role in the body's lipid metabolism. In the current study, LL-Xupu geese have increased the mRNA expression level of FABP4, ACAT2, and FASN compared to SL-Xupu geese. To our knowledge, there are few researches on the liver size of Xupu geese. Indeed, it is widely accepted that Landes geese is the optimal breed for its high yield of fatty liver in the world, which contributed to the profitability of production. The current study showed that fatty liver of Xupu geese will be beneficial for human health and Xupu geese also has the capacity of producing high-quality Foie gras.

Conclusions

In summary, the variation pattern of the liver weight conformed to a normal distribution in overfed Xupu geese. The different susceptibility to fatty liver of overfed Xupu geese was closely associated with the different plasma TG, VLDL concentration, CHE, LPS, and LPL activities. Meanwhile, the regulation of the FABP4, SCD, FADS, ELOVL1, ACAT2, and FASN gene expression in liver induced may be associated with hepatic steatosis, which may be the result of significant difference in liver size of overfed Xupu geese.

Disclosure statement

No potential conflict of interest was reported by the authors.

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