A Study on the Anti-obesity Effect of Caffeoylquinic Acids From the Cell Culture of Saussurea Involucrata

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A study on the anti-obesity effect of caffeoylquinic acids from the cell culture of *Saussurea involucrata*

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Abstract

Backgroud: *Saussurea involucrata* Kar. et Kir. (Compositae) (CCSauI) cells are rich in caffeoylquinic acids (CQAs), which have plasma lipid reducing properties and anti-obesity effects, although the mechanisms remain unclear. Clarify CQA's anti-obesity mechanism and provide new treatments for obesity.

Methods: Sprague Dawley (SD) rat were fed a high-fat diet (HFD), then CQAs was intragastric administrated (0.1, 0.5 or 1 mg/mL). Rats were randomly divided into five groups (n=30): NC, MC, TPL (0.1 mg/mL), TPM (0.5 mg/mL) and TPH (1 mg/mL). Digestive enzyme inhibition was obtained by measuring the inhibition rate of α-amylase, α-glucosidase, and pancreatic lipase *in vitro*. Anti-obesity function was detected by determining the content of total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), malondialdehyde (MDA) superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px). To analyze the related mechanisms qRT-PCR was employed.

Results: From *in vitro* enzyme activity assays, the IC_{50} values of the CQAs extract for α-amylase, α-glucosidase, and lipase were 0.631, 0.31, and 0.438 mg/mL, respectively. CQAs administration for 8 weeks decreased retroperitoneal fat and serum and liver TC, TG, LDL-C, and MDA. Comparatively, levels of HDL-C, SOD, and GSH-Px were increased. Real-time fluorescent quantitative PCR showed inhibited expression of fatty acid synthase and 3-hydroxy-3-methyl glutaryl and coenzyme A reductase, peroxisome proliferator-activated receptor-α activation, and 7α-hydroxylase

promotion.

Conclusion: This study explored the role of CQAs in inhibiting digestive enzyme activities and up-regulating the expression of lipase, significant for prevention and treatment of diabetes and obesity.

Keywords: lipid metabolism; digestive enzymes; real-time fluorescence quantitative PCR

Background

Saussurea involucrate Kar. *et* Kir. (Compositae), a cultivar used in traditional Chinese medicine, grows at high altitudes where snow covers cliffs and ice protects rock crevices all year round. Its growth environment is harsh and its life cycle is long. It takes 5 to 6 years to blossom and bear fruit under natural conditions. Additionally, because *S. involucrata* is subject to private indiscriminate harvesting and its artificial cultivation is very difficult, it is a rare and precious traditional Chinese medicine resource (Fu et al. 2006; Chik et al. 2015). In recent years, *S. involucrata* has been the topic of extensive research at home and abroad. Using knowledge of its effective ingredients and medicinal efficacy, researchers have conducted artificial propagation, used tissue and cell culture to obtain the active components, and biotechnology to develop substances to replace those found in wild *S. involucrata* (Jia et al. 2005; Guo et al. 2007; Jia & Wu 2008). The cell culture of *S. involucrata* (CCSauI) is expected to replace the use of wild *S. involucrata* in the fields of medicine and health food.

Obesity has become a major societal health problem and many attempts to manage obesity have little effect. The extraction of active substances from natural plants and the investigation of their anti-obesity properties in an effort to identify a treatment to prevent and reduce body weight has become a research area of increasing interest (Rezvani et al. 2018; Kim et al. 2019).

At present, Caffeoylquinic acids (CQAs) have been isolated from many plants, including those with higher content such as sweet potato leaves, coffee, day lilies, honeysuckle, tea, sunflower seeds, cotton fungus, and *S. involucrata* (Azuma et al. 2000; Hung et al. 2006; Wang et al. 2014; Indy Tamayose et al. 2019). CQAs reduce plasma lipids and possess an anti-obesity effect (Nishimura et al. 1999; Cho et al. 2010), but the mechanism is still unclear.

In this study, CCSauI was used as the experimental material to extract and purify CQAs by macroporous adsorption resin and preparative liquid chromatography (Negishi et al. 2004; Sithisarn et al. 2011; Zou et al. 2014), and the anti-obesity effect of CQAs was preliminarily studied. Using rats fed a high-fat diet (HFD), the ability of CQAs to inhibit digestive enzymes *in vitro* was investigated to provide a theoretical basis for the development and application of *S. involucrata* as a source of natural anti-obesity products.

Methods

Main reagents

CCSauI was developed and cultivated by the National and Local Joint

Engineering Center for Rare and Endangered Medicinal Plants (Dalian, China). The product was stored at Dalian Practical Biotechnology Co., Ltd. (Dalian, China), and the product identification was 20166. Chlorogenic acid, HP-20 large adsorbent resin, porcine pancreatic α-amylase, α-glucosidase and porcine pancreatic lipase were obtained from Shanghai Yuanye Biotechnology Co., Ltd. (Shanghai, China). The total cholesterol (TC) kit, triglyceride (TG) kit, high- and low-density lipoprotein cholesterol (HDL-C, LDL-C) kit, superoxide dismutase (SOD) kit, malondialdehyde (MDA) kit, glutathione peroxidase (GSH-Px) kit, and Coomassie blue protein kit were obtained from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). The RNA extraction kit, reverse transcription kit and real-time PCR kit were obtained from Nanjing Vazyme Biotech Co., Ltd. (Nanjing, China).

Instruments and equipment

The nucleic acid protein detector (model number HD-4) was from Shanghai Qingpu Huxi Instrument Factory, the rotary evaporator (model number N-1210BV-W) was from Tokyo Rikakikai Co. Ltd. (Tokyo, Japan), the microplate reader (model number S/N 415-2903) was from BMG Company (Offenburg, Germany), the vacuum freeze dryer (model number Epsilon 2-4 LSCplus) was from Marin Christ Company, the BUCHI preparative liquid chromatography system (model number C-660) was from BUCHI Ltd. (Flawil, Switzerland) and the real-time PCR machine (model StepOnePlus) was from Thermo Fisher Scientific.

Extraction of caffeoylquinic acids

The CQAs were obtained by accurately weighing 10 g of CCSauI powder into a conical flask, adding 150 mL of boiling deionized water (the ratio of material to liquid was 1:15), and incubating the solution in a 95℃ constant temperature water bath for 0.5 to 1 h with shaking. The extracted solution was centrifuged at 5000 *g* for 10 min to obtain the supernatant, which was then subjected to rotary evaporation and concentration. The resulting material was freeze dried at -80℃ to obtain the crude extract powder.

The crude extract of CCSauI was diluted with deionized water to 10 g/L in a total volume of 500 mL. The sample was applied to a HP-20 macroporous adsorption resin chromatography column (45 × 450 mm; column bed 300 mL) at a constant speed of 1 mL/min. The sample was eluted by successively washing the column with four column volumes of distilled water and two column volumes of 70% ethanol at 2.0 mL/min. A HD-4 nucleic acid protein detector was used to check the absorbance value of the eluent at 280 nm. The collected eluent was concentrated and freeze-dried to obtain a relatively pure powder of CQAs.

The obtained crude extract powder (1 g) was prepared with deionized water at 5 g/L in a total volume of 200 mL for the liquid phase purification. The sample (5 mL) was applied to a C18 spherical chromatographic column (particle size 20-35 μm), and eluted at 1.5 mL/min with a gradient (0-20 min, 12% A-30% A; 20-50 min, 30% A-50% A; 50-55 min, 50% A-88% A; 55-60 min, 88% A plus 0.5% formic acid [phase A]) and acetonitrile (phase B) with detection at 326 nm. The substances that eluted at the same time as the chlorogenic acid standard were collected for rotary evaporation,

frozen, and vacuum concentrated to yield the purified extract powder.

Inhibitory effect on digestive enzymes *in vitro*

The ability of the purified extract powder to inhibit porcine pancreatic α-amylase, α-glucosidase, and porcine pancreatic lipase was determined using specific enzyme kits (Liu et al. 2020; Navarro J et al. 2020).

Animals

A total of 150 specific pathogen free (SPF) Sprague Dawley (SD) rats (3 weeks old approximately 60 g each) were randomly divided into the following five groups (30 rats per group), blank control (NC), model control (MC), gavage of low dose CQAs (0.1 mg/mL) CQAsfrom CCSauI (TPL), gavage of medium (0.5 mg/mL) dose CQAs from CCSauI (TPM) and gavage of high dose (1 mg/mL) CQAs from CCSauI (TPH) （Figure 1）. The NC group was fed a normal diet while the other groups were fed an HFD. Each group had access to 50 g of feed per day and 2 mL CQAs aqueous solution was delivered by gavage to three of the five groups (Table 1). The rats were kept in a humidity-controlled room on a 12- h light/dark cycle with food and water available *ad libitum* for the breeding period. The amounts of drinking water and food intake were recorded every day, and the weight of rat was recorded every week.

Determination of the physiological indexes of the rats

The rats were weighed before sacrifice. After blood was collected from their

eyeballs, anesthetic was injected, then the rats were killed by cervical dislocation, and their body and tail lengths were measured. After labeling the rats, the liver was examined, dissected, washed with PBS and weighed. The liver was cut into small pieces and stored at -80℃. The serum extracted from the blood was stored at -80℃. Lee's physiological index was calculated as follows.

Lee's index (g/cm) = body weight 1/3 (g) × 133/body length (cm)

Epididymal fat index = epididymal fat weight (g)/rat body weight (g) × 100

Biochemical analysis of rat serum

The levels of TC, TG, HDL-C, LDL-C SOD, and GSH-Px in the rat serum and liver were measured according to the specific experimental methods provided for each kit.

Data analysis

Duncan's test ($P < 0.05$) provided by IBM SPSS 22.0 statistical analysis software was used to analyze the significance of the sample differences.

Expression level detection by real-time fluorescence quantitative PCR

Using the liver stored at -80℃, a hepatocyte homogenate was prepared with a cell breaker. The RNA was extracted from the hepatocyte homogenate, and the cDNA was transcribed and amplified according to the manufacturers' instructions for the RNA extraction kit, reverse transcription kit and real-time PCR kit. The specific

information of the primers is shown in Table 2. The primers were synthesized at Sangon Biotech (Shanghai) Co., Ltd. The cycle threshold value for each key enzyme gene of lipid metabolism was measured to ensure that the PCR signal had reached the required threshold and then, the gene expression level of each key enzyme of lipid metabolism was calculated using the $2^{-\Delta\Delta CT}$ method.

Results

Extraction and purification of caffeoylquinic acids

The crude extract of CCSauI was purified using HP-20 macroporous adsorption resin. An aqueous solution of the extracted material was prepared and the CQAs in the crude extract were further purified using column chromatography. As shown in Figure 2, the preparative liquid phase diagram (A) of substances eluted from the HP-20 macroporous adsorption resin was basically the same as those of the preparative liquid phase diagram (B) of the chlorogenic acid standard, and it was concluded that the substances in the two samples were essentially the same. Thus, the extracted and purified substances were chlorogenic acids and their derivatives.

Inhibitory effect of caffeoylquinic acids on digestive enzymes *in vitro*

α-Amylase, α-glucosidase, and pancreatic lipase play an important role in regulating the body's sugar metabolism and lipid metabolism. α-Amylase digests and decomposes the α-1,4-glycosidic bonds in carbohydrates such as starch, and helps the body absorb sugar, which is converted into blood sugar and glycogen. α-Glucosidase

helps the body digest disaccharides and convert them into monosaccharides, thereby promoting the body's absorption of sugar. Pancreatic lipase decomposes triglycerides in foods into monoacylglycerols and free fatty acids. After being absorbed by the body, triglycerides are synthesized and accumulate in the body, leading to obesity. The inhibitory effect of CQAs extracted and purified from CCSauI on the activities of α-amylase, α-glucosidase, and porcine pancreatic lipase was determined. It was found that the inhibitory effect of CQAs on digestive enzyme activity was obvious, and the inhibitory effect increased with increasing concentration of CQAs. The IC_{50} values for the CQAs extract for α-amylase, α-glucosidase, and lipase were 0.631, 0.31, and 0.438 mg/mL, respectively. When the final concentration of the sample solution reached 2.25, 1.5, and 1.5 mg/mL, the inhibition of α-amylase, α-glucosidase, and lipase reached 95.86%, 95.38%, and 97.1%, respectively. The results are shown in Figure 3.

Body weight and Lee's index of rats

The weight change in rats fed a HFD for 8 weeks is shown in Figure 4. The weight of rats in the MC group was 23% higher than that of the NC group, and indicated the formation of obese rats. The weights of the TPL, TPM and TPH groups were 7.5%, 12.5%, and 17.5% lower than those in the MC group.

The dissected livers are shown in Figure 5. A typical liver of rats from the NC group was dark red, with a smooth surface, sharp edges, and elastic when pressed with fingers. The liver of rats in the MC group were mostly yellow and white, with

diffuse surfaces, thick and blunt edges, and tissue adhesion. The liver color tended to be normal for the TPL, TPM and TPH groups. Lee's index is an index of obesity that takes into account the weight and body length of the rats and is an effective index to evaluate the degree of obesity. In general, the larger the Lee's index, the more obese the animal is. As shown in Figure 6, the Lee's index of the rats from the 6^{th}, 7^{th}, and 8^{th} week model groups and each dose group were significantly different from that of the blank group. Additionally, the Lee's index of each dose group was significantly lower than that of the model group.

Rat liver and epididymal fat indexes

The liver weight index and epididymal fat index of the rats showed similar trends as the Lee's index (Figure 7). Compared with the NC group, the rat liver and epididymal fat indexes of the MC and each dose group from the 6^{th}, 7^{th}, and 8^{th} weeks were significantly increased, and the differences were extremely significant. Compared with the MC group, the TPL, TPM, and TPH groups were significantly lower, and the differences between the groups were significant. The TPH group had a significantly lower epididymal fat index compared with the MC group. The epididymal fat index of the TPH group was similar to that of the NC group, which indicates that CQAs can effectively increase the rat liver index and epididymal fat index of rats fed a HFD, and may have an anti-obesity effect.

Changes of biochemical indexes in rats

TC is an important reference index for hyperlipidemia and atherosclerosis and represents the total level of cholesterol in the blood and liver homogenate. As shown in Figure 8, after 8 weeks, the TC levels of rats from the TPL, TPM, and TPH groups were significantly different from that of the MC group ($P < 0.05$). At the 6^{th} week, the TC levels were 5.3%, 14.8%, and 19.3% lower than that of the MC group, at the 7^{th} week, they were 6.9%, 16.3%, and 20.4% lower than that of the MC group, and at the 8^{th} week, they were 8.8%, 19.7%, and 23.1% lower than that of the MC group.

TG is the most common fat in the body. It is primarily present in the blood and liver and is closely related to the risk of atherosclerotic cardiovascular disease. The change in the trend of serum TG level in each group was consistent with those of the TC level (Figure 9). The TG levels in the TPL, TPM, and TPH groups were 18.2%, 32.3%, and 37.4% lower than that of the MC group at the 8^{th} week. When the TG values of rats at the 6^{th} and 7^{th} week were compared, time-dependent and dose-dependent effects were observed for the changes in the TG levels.

HDL-C reflects the amount of high-density lipoprotein in the body's serum and liver. In specific clinical applications, high-density cholesterol can prevent atherosclerosis and reduce obesity and its complications. The levels of HDL-C in the TPL, TPM, and TPH groups were significantly higher than that of the MC group ($P < 0.01$), which increased by 16.7%, 36.1%, and 50% at the 6^{th} week. In the 7^{th} week, the HDL-C increased by 16.9%, 56.3%, and 70.4%, and in the 8^{th} week, it increased by 12.7%, 40.5%, and 58.2%, for the TPL, TPM, and TPH groups, respectively (Figure 10).

LDL-C reflects the index level of low-density lipoprotein in the body's serum and liver and is the primary lipoprotein that causes atherosclerotic diseases in animals. The change in the LDL-C level in serum was also consistent with the change in the TC level. The LDL-C levels in the TPL, TPM, and TPH groups decreased by 17.9%, 40.4%, and 66.3% at the 6th week, 22.1%, 40.0%, and 65.3% at the 7th week, and 22.2%, 40.4%, and 61.6% at the 8th week compared with that of the MC group (Figure 11).

SOD, also known as liver protein, can eliminate excess oxygen free radicals in the collective tissue, inhibit the synthesis of lipid peroxides, and prevent atherosclerosis to a certain extent. The SOD levels in the TPL, TPM, and TPH groups increased by 16.5%, 25.1%, and 35.1% at the 6th week, 15.5%, 21.9%, and 43.5% at the 7th week, and 19.6%, 25.1%, and 46.9% at the 8th week compared with that of the MC group (Figure 12).

GSH-Px can effectively remove excessive free radicals in the body and prevent the production of lipid peroxides, thereby protecting the liver and producing an anti-obesity effect. The GSH-Px levels in the TPL, TPM, and TPH groups increased by 17.7%, 36.8%, and 43.9% at the 6th week, 17.3%, 36.4%, and 41.7% at the 7th week, and 18.4%, 37.8%, and 43.5% at the 8th week compared with that of the MC group (Figure 13).

MDA can directly reflect the degree of lipid peroxidation in the body, and it is also a common indicator for the clinical detection of obesity. The MDA levels in the TPL, TPM, and TPH groups decreased by 18.6%, 36.4%, and 61.4% at the 6th week,

18.3%, 39.6%, and 59.4% at the 7th week, and 25.9%, 42.3%, and 60.6% at the 8th week compared with that of the MC group (Figure 14). The change trend of each biochemical index in the liver was similar to those in the serum (Figure S1-S7).

Compared with the MC group, CQAs can effectively reduce the levels of TG, TC, and LDL-C in the serum and liver homogenate of HFD rats, and increase the level of HDL-C, which indicates that CQAs may be used to regulate disorders of blood lipid metabolism, which in turn, may prevent the occurrence of obesity diseases such as atherosclerosis. At the same time, CQAs can significantly increase the levels of SOD and GSH-Px in the serum and liver homogenate of HFD rats. It also reduces the level of MDA in rats, which indicates that CQAs can increase the level of endogenous antioxidant enzyme activity in rats, inhibit the generation of lipid peroxides, and thus have an anti-obesity effect. However, as shown by the trend of each index, the effect of time varies depending on the dose group, and the dose of CQAs has a more obvious anti-obesity effect than time. However, in the case of the same dose, the changes in the various indicators of the experimental group over time were not significant. It may be due to the continuous feeding of HFD while gavage during the experiment, which makes the results show a certain time dependence. This shows that in the process of fat loss, a reasonable diet is also very important.

Gene expression levels of adipose metabolic enzymes in rats

As shown in Figure 15, the fatty acid synthase (*FAS*) gene expression levels from the MC model group, and the TPL, TPM and TPH experimental groups were 1.39,

1.32, 1.26, and 1.14 times higher than that of the NC group, respectively. The expression levels of the lecithin-cholesterol acyltransferase (*LCAT*) gene were 1.03, 1.02, 1.06, and 1.11 times that of the NC group, and the expression levels of the 3-hydroxy-3-methyl glutaryl coenzyme A reductase *(HMG-COA*) gene were 1.21, 0.9, 0.91, and 0.72 times that of the NC group, respectively. The expression levels of the cholesterol 7α-hydroxylase (*CYP7A1*) gene were 1.98, 2.12, 2.78, and 3.21 times higher than that of the NC group, and the expression levels of peroxisome proliferator-activated receptor-α (*PPARα*) gene were 0.83, 0.96, 1.09, and 1.23 times higher than that of the NC group, respectively. These results showed that CQAs significantly up-regulated the expression levels of the *CYP7A1* and *PPARα* genes and significantly down-regulated the expression levels of the *FAS* and *HMG-COA* genes, while the expression level of the *LCAT* gene showed no obvious upward trend. Taken together, the CQAs in CCSauI inhibited the expression of the *FAS* and *HMG-COA* genes, activated the expression of the *PPARα* gene, and promoted the expression of the *CYP7A1* gene, which further suggests that they had an anti-obesity effect.

Discussion

In recent years, the global obesity rate has shown a linear growth trend. Obesity not only affects the quality of life and body beauty of patients, but also seriously harms their health (Ravussin & Bogardus 1992; McCafferty et al. 2020). The causes of obesity include active mRNA expression of genes related to adipose formation, which affects the differentiation of adipose cells. Additionally, the activities of

enzymes related to adipose metabolism are affected by obesity, which may slow the degradation of adipose and increase adipose cell volume, cell number, and adipose accumulation (Spiegelman & Flier 1996). Therefore, it is of great significance to study the inhibition of digestive enzymes and the promotion of adipose metabolic enzyme expression for the prevention and treatment of obesity metabolic diseases.

The accumulation of adipose in skeletal muscle, kidney, pancreas, heart, and other target organs, especially visceral adipose, is closely related to metabolism, and may cause obesity-related diseases. Shimoda *et al*. (2006) found that chlorogenic acid could effectively reduce liver adipose content. Cho *et al*. (2010) found that chlorogenic acid could increase the content of leptin, significantly reduce the weight of obese mice and the levels of TC, TG, and LDL-C in serum, and significantly improve the level of HDL-C in serum. Rodriguez de Sotillo and Hadley (2002) proposed that in chlorogenic acid-treated rats, fasting plasma cholesterol and triacylglycerol concentrations significantly decreased as well as the liver triacylglycerol concentrations. Chlorogenic acid has also been shown to regulate glucose-6-phosphatase involvement in glucose metabolism (Hemmerle et al. 1997) and reduce the risk of cardiovascular disease by reducing LDL-C and TC. Chlorogenic acid also inhibited the growth of preadipocytes to achieve an anti-obesity effect (Hsu et al. 2006; Alonso-Castro et al. 2008). These observations for chlorogenic acid were consistent with the experimental results of this study. CQAs extracted from the culture of *S. involucrata* inhibited the activity of digestive enzymes and the formation of fatty liver. It was also found that CQAs inhibited the expression of *FAS*

and *HMG-COA*, activated *PPARα*, and promoted the expression of *CYP7A1*, thereby having an anti-obesity effect.

Conclusion

CQAs had an anti-obesity effect on rats fed a HFD. This effect was achieved by inhibiting the activity of digestive enzymes and up-regulating the expression level of adipose metabolic enzymes. The resulting changes in the level of plasma adipokines and body fat distribution helped to reduce and down-regulate the biosynthesis of fatty acids and cholesterol, thus achieving an anti-obesity effect. The production of anti-obesity products using CCSauI as the primary culture may provide a way to treat obesity and achieve economic benefits by reducing health care costs.

Declarations

Ethics approval and consent to participate

The experimental protocol was established according to the ethical guidelines of the Helsinki Declaration and was approved by the Human Ethics Committee of Jilin Agricultural University. All efforts were made to minimize animal suffering.

Availability of data and material

All data generated or analyzed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Bao Changjie and Gao Junpeng: Validation. Wang Peng: Resource. Zhang Sitong Writing - review & editing. Chen Guang: Experimental design. All authors read and approved the final manuscript.

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Footnotes

Figure captions

Figure 1 Treatment methods of rats in each group

Figure 2 Liquid chromatogram of the chlorogenic acid standard preparation (A) and the HP-20 elution sample (B)

Figure 3 Inhibition of digestive enzymes *in vitro* by caffeoylquinic acids obtained from a cell culture of *Saussurea involucrata*

Figure 4 Body weight change of 8-week-old rats fed a high-fat diet in the presence of caffeoylquinic acids obtained from a cell culture of *Saussurea involucrata*

Figure 5 Photos of the anatomical liver of rats fed a high-fat diet in the presence of caffeoylquinic acids obtained from a cell culture of *Saussurea involucrata* after 8 weeks of treatment

Figure 6 Lee's index of rats fed a high-fat diet in the presence of caffeoylquinic acids obtained from a cell culture of *Saussurea involucrata* for 6, 7, and 8 weeks

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Figure 9 Changes of TG content in the serum of rats fed a high-fat diet in the presence of caffeoylquinic acids obtained from a cell culture of *Saussurea involucrata* for 6, 7, and 8 weeks

Figure 10 Changes of HDL-C content in the serum of rats fed a high-fat diet in the presence of caffeoylquinic acids obtained from a cell culture of *Saussurea involucrata* for 6, 7, and 8 weeks

Figure 11 Changes of LDL-C content in the serum of rats fed a high-fat diet in the presence of caffeoylquinic acids obtained from a cell culture of *Saussurea involucrata* for 6, 7, and 8 weeks

Figure 12 Changes of SOD content in the serum of rats fed a high-fat diet in the presence of caffeoylquinic acids obtained from a cell culture of *Saussurea involucrata* for 6, 7, and 8 weeks

Figure 13 Changes of GSH-Px content in the serum of rats fed a high-fat diet in the presence of caffeoylquinic acids obtained from a cell culture of *Saussurea involucrata* for 6, 7, and 8 weeks

Figure 14 Changes of MDA content in the serum of rats fed a high-fat diet in the presence of caffeoylquinic acids obtained from a cell culture of *Saussurea involucrata* for 6, 7, and 8 weeks

Figure 15 Gene expression levels of lipid metabolism enzymes in the liver of rats fed

a high-fat diet in the presence of caffeoylquinic acids after 8 weeks of treatment

Table captions

Table 1 Grouping of rats used in this study

Table 2 Primer and probe sequence design of key lipid metabolism genes examined in this study

Figures

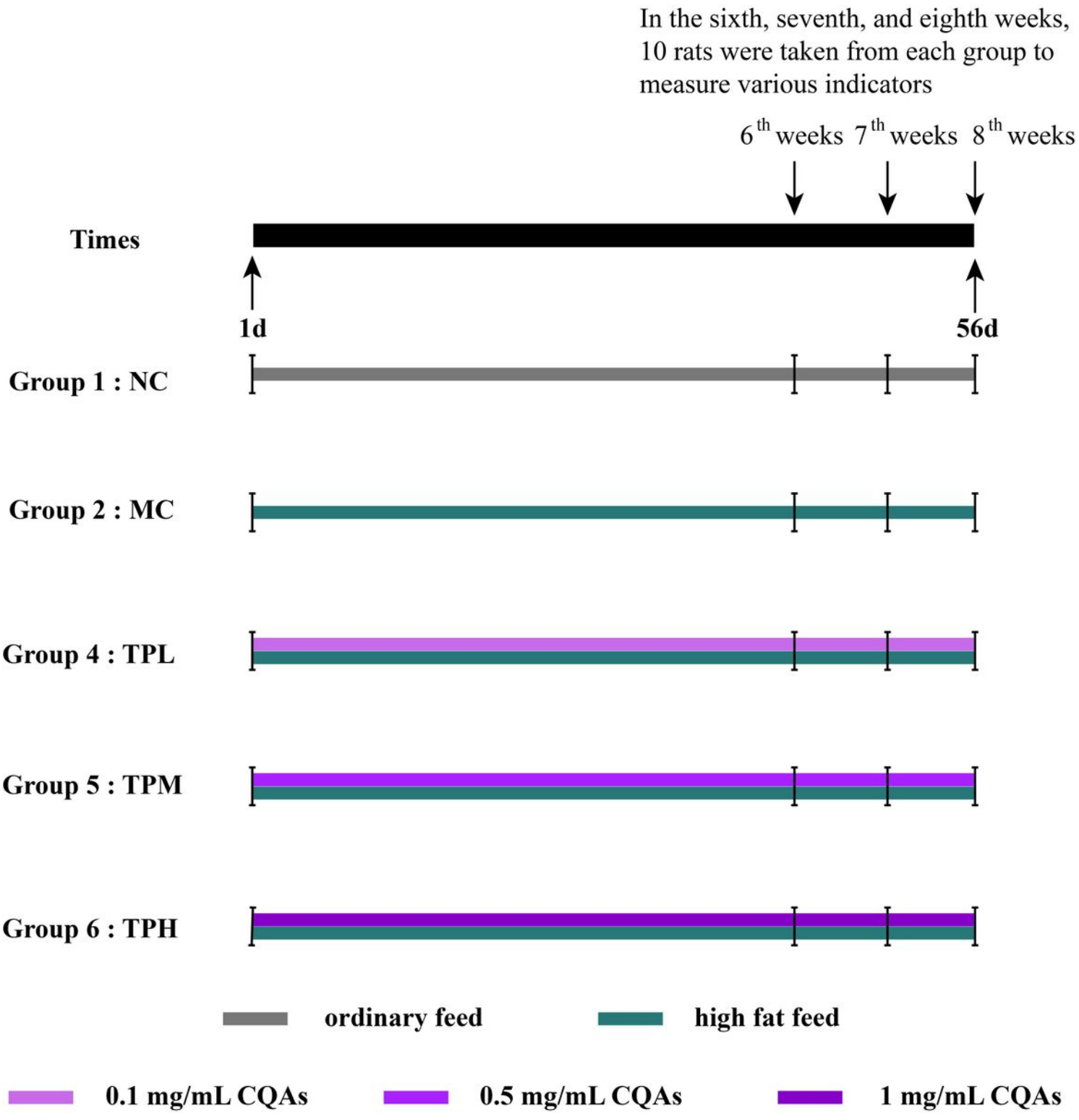
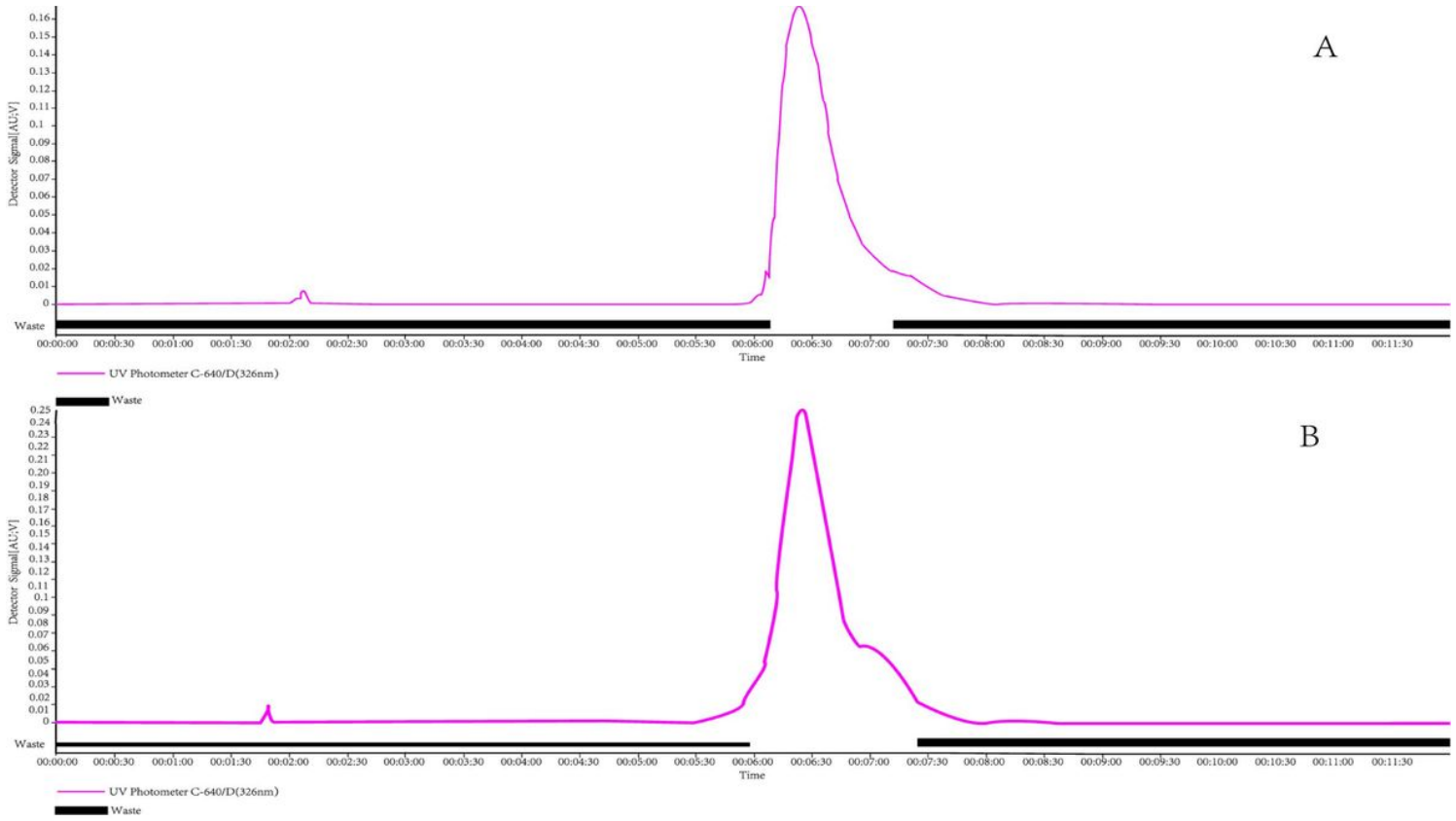


Figure 1

Treatment methods of rats in each group



Note: Panel A shows the liquid phase diagram of the chlorogenic acid standard preparation, and panel B shows the liquid phase diagram of the HP-20 elution sample.

Figure 2

Liquid chromatogram of the chlorogenic acid standard preparation (A) and the HP-20 elution sample (B)

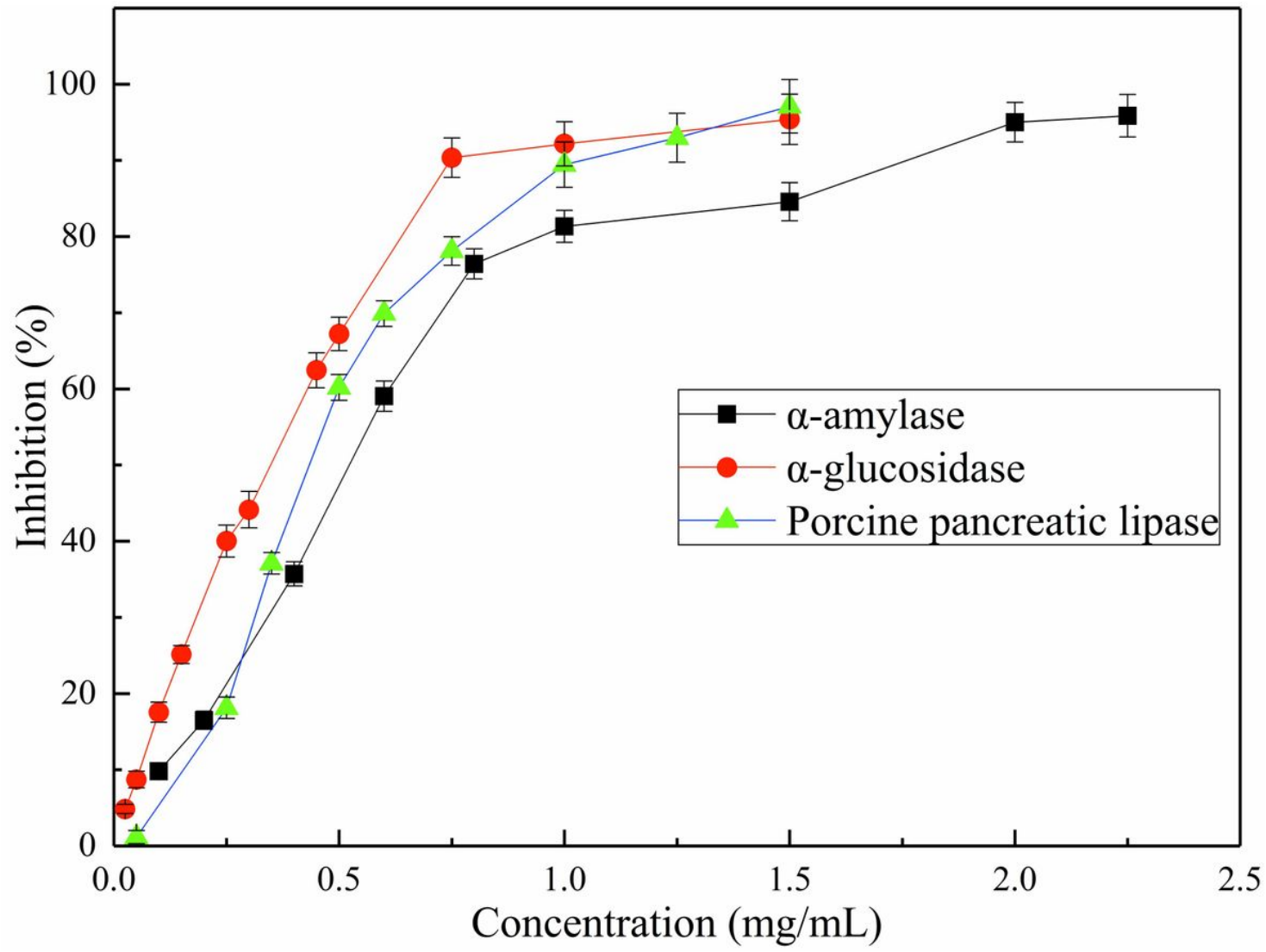


Figure 3

Inhibition of digestive enzymes in vitro by caffeoylquinic acids obtained from a cell culture of Saussurea involucrata

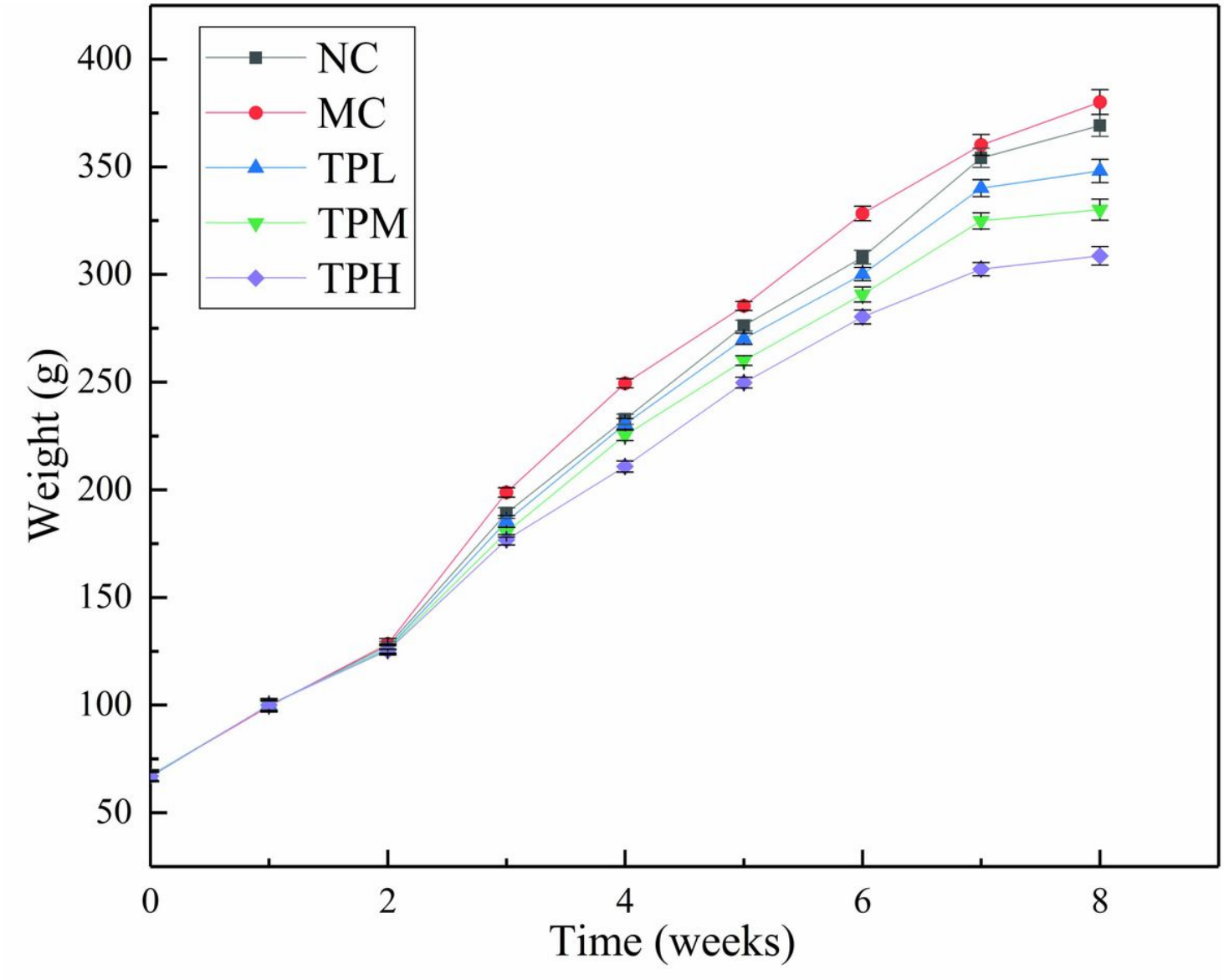


Figure 4

Body weight change of 8-week-old rats fed a high-fat diet in the presence of caffeoylquinic acids obtained from a cell culture of Saussurea involucrata

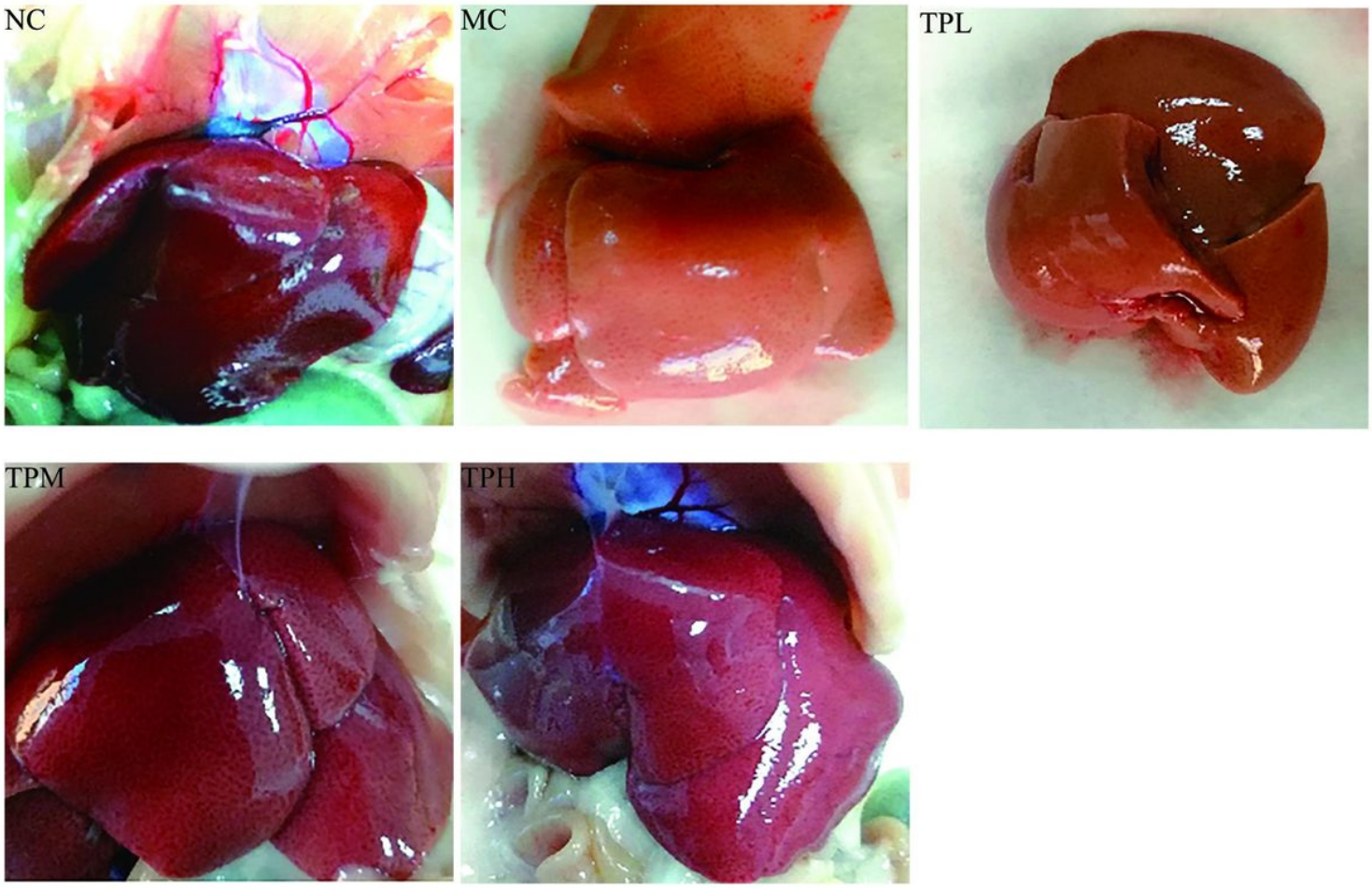


Figure 5

Photos of the anatomical liver of rats fed a high-fat diet in the presence of caffeoylquinic acids obtained from a cell culture of Saussurea involucrata after 8 weeks of treatment

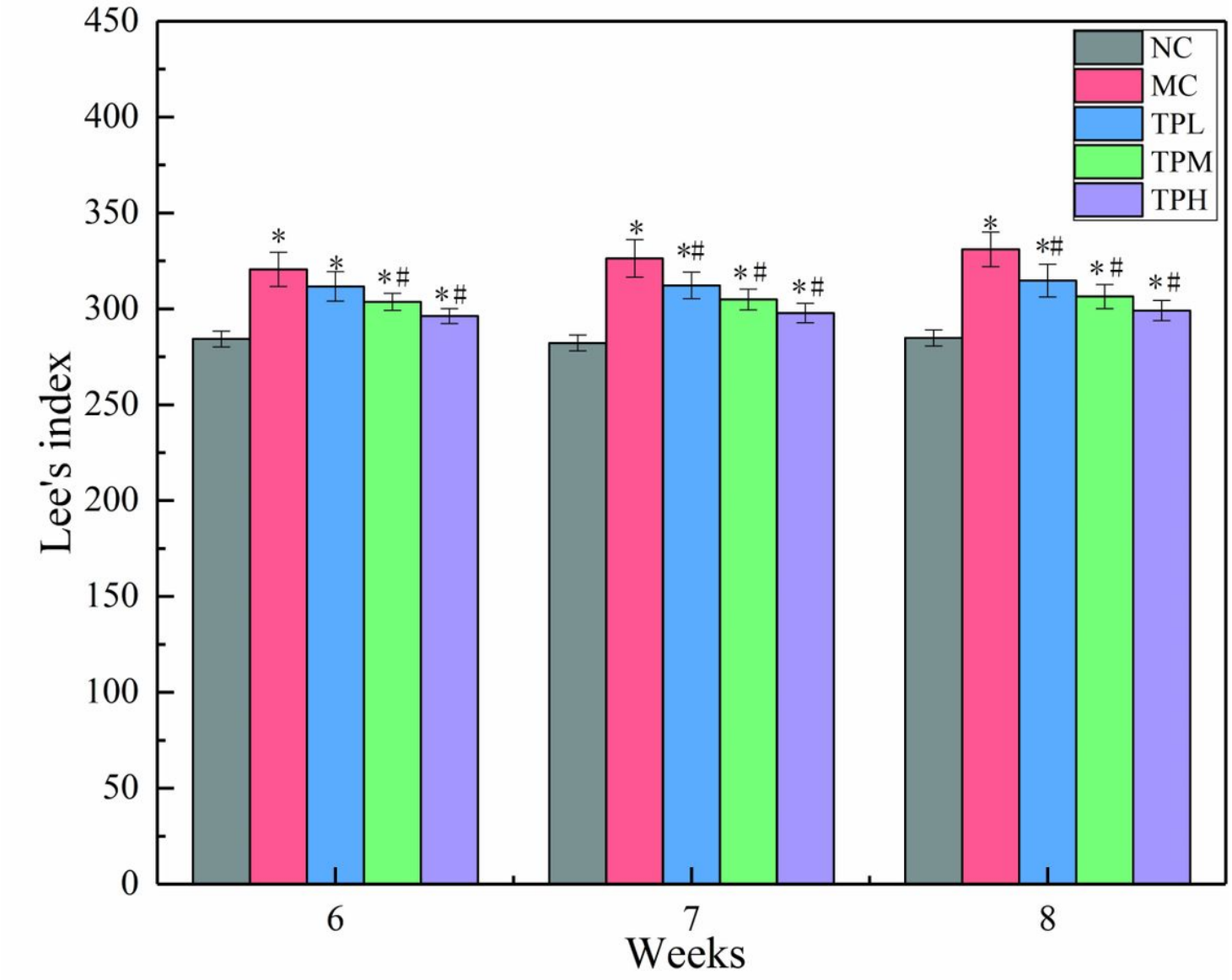


Figure 6

Lee's index of rats fed a high-fat diet in the presence of caffeoylquinic acids obtained from a cell culture of Saussurea involucrata for 6, 7, and 8 weeks Note: * Compared with NC, P value < 0.05. # Compared with MC, P value < 0.05.

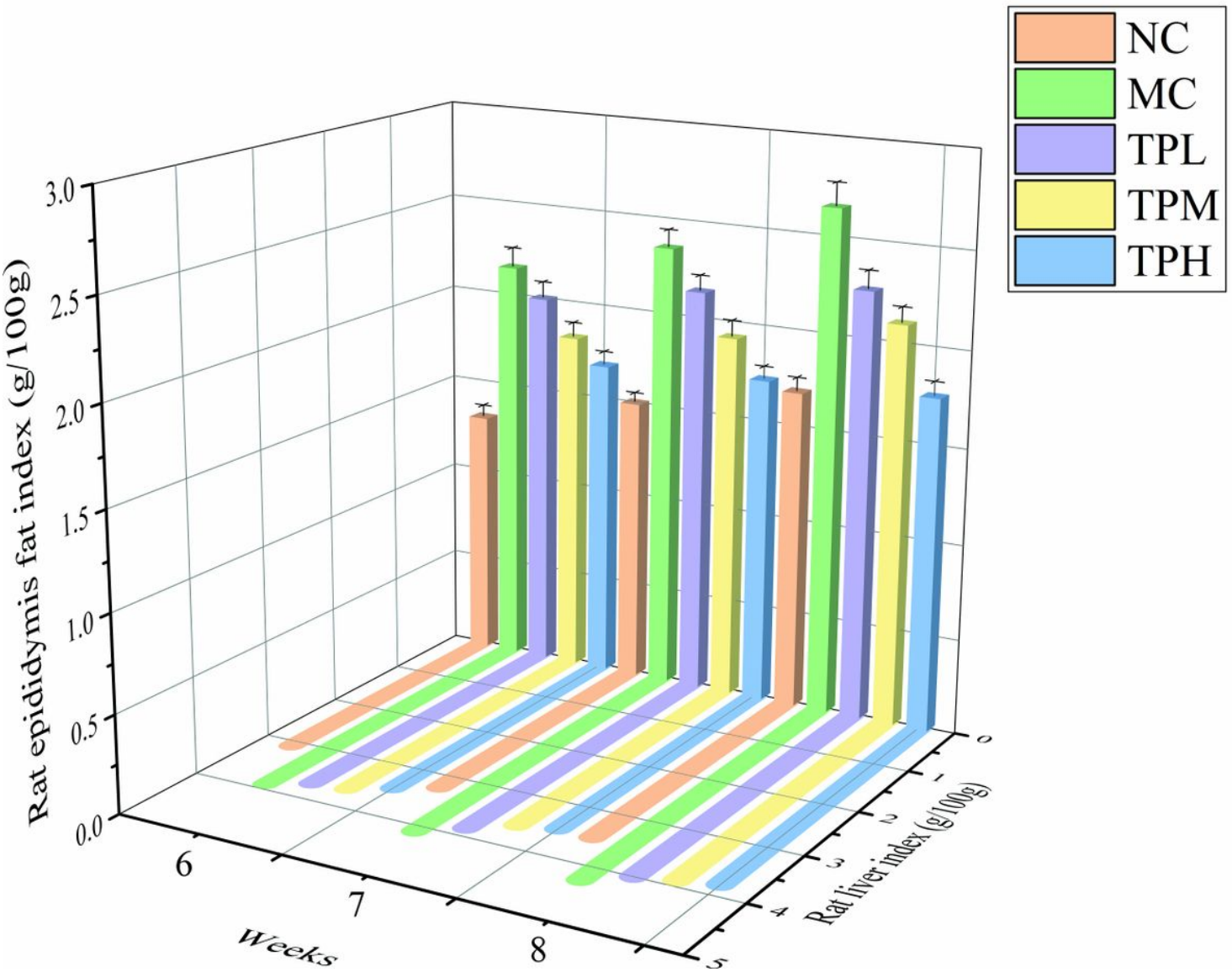


Figure 7

Liver and epididymal fat indexes of rats fed a high-fat diet in the presence of caffeoylquinic acids obtained from a cell culture of Saussurea involucrata for 6, 7, and 8 weeks

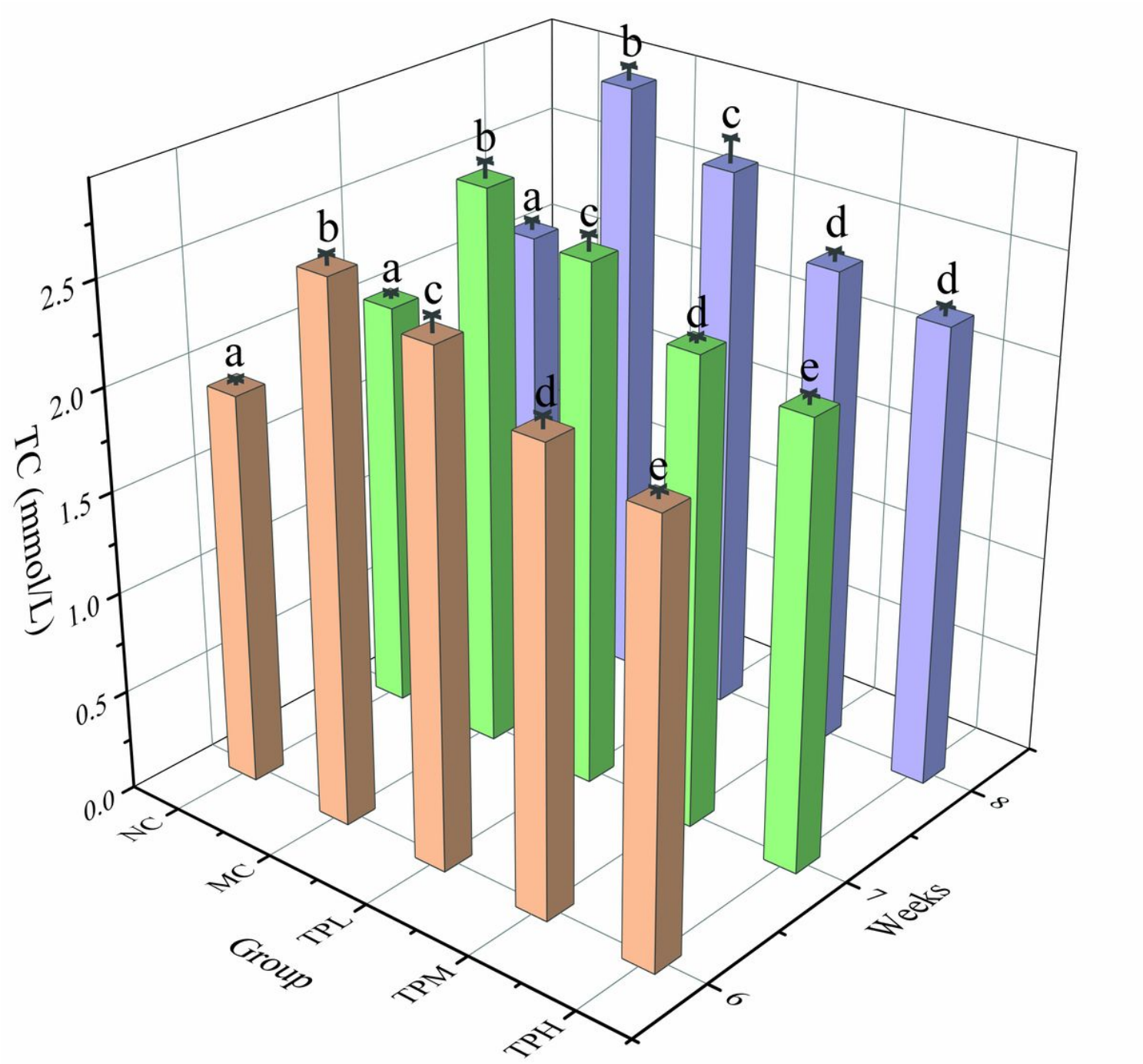


Figure 8

Changes of TC content in the serum of rats fed a high-fat diet in the presence of caffeoylquinic acids obtained from a cell culture of Saussurea involucrata for 6, 7, and 8 weeks Note: In the same week, the same word means P value > 0.05, the different word means P value < 0.05.

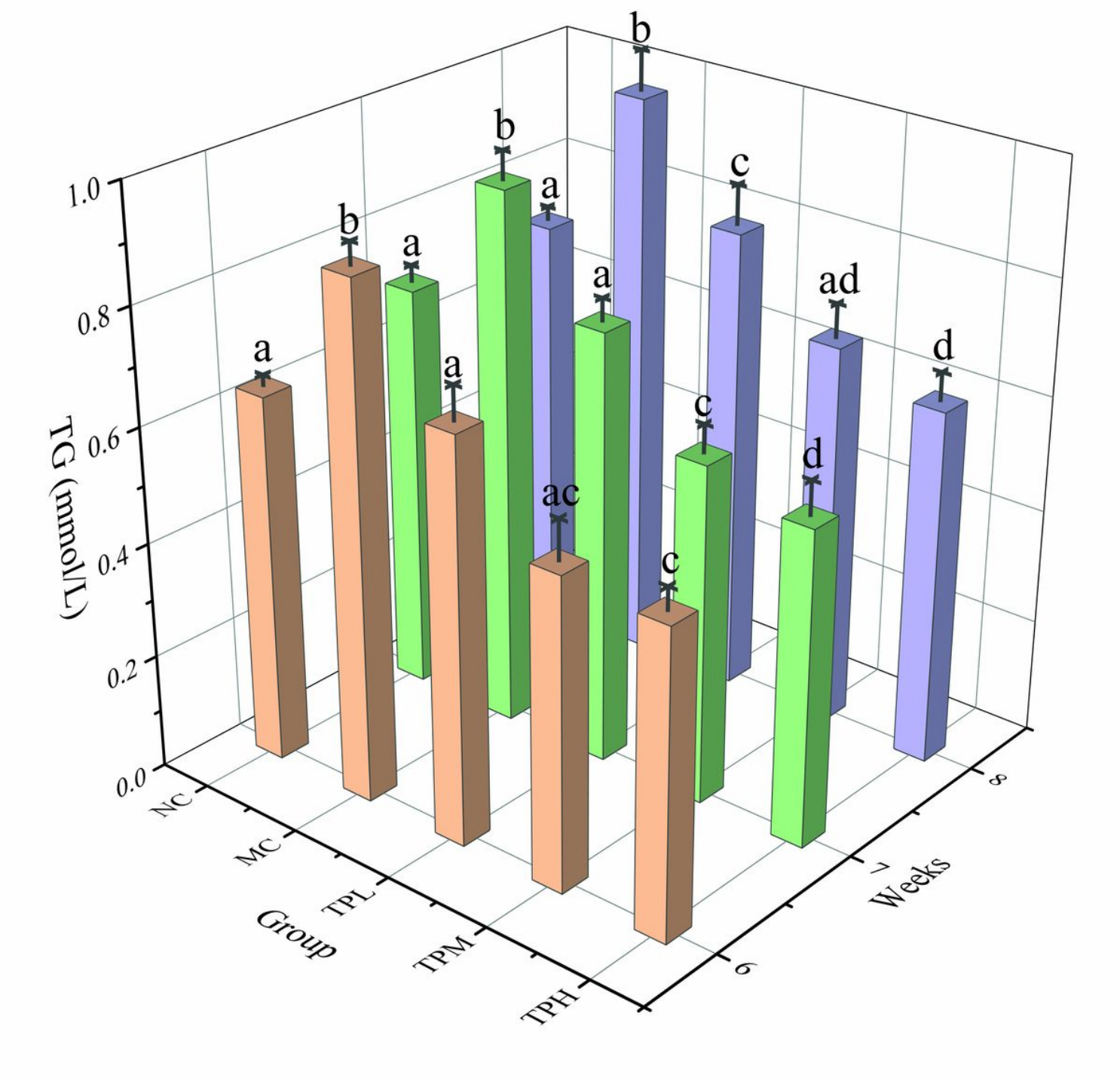


Figure 9

Changes of TG content in the serum of rats fed a high-fat diet in the presence of caffeoylquinic acids obtained from a cell culture of Saussurea involucrata for 6, 7, and 8 weeks Note: In the same week, the same word means P value > 0.05, the different word means P value < 0.05.

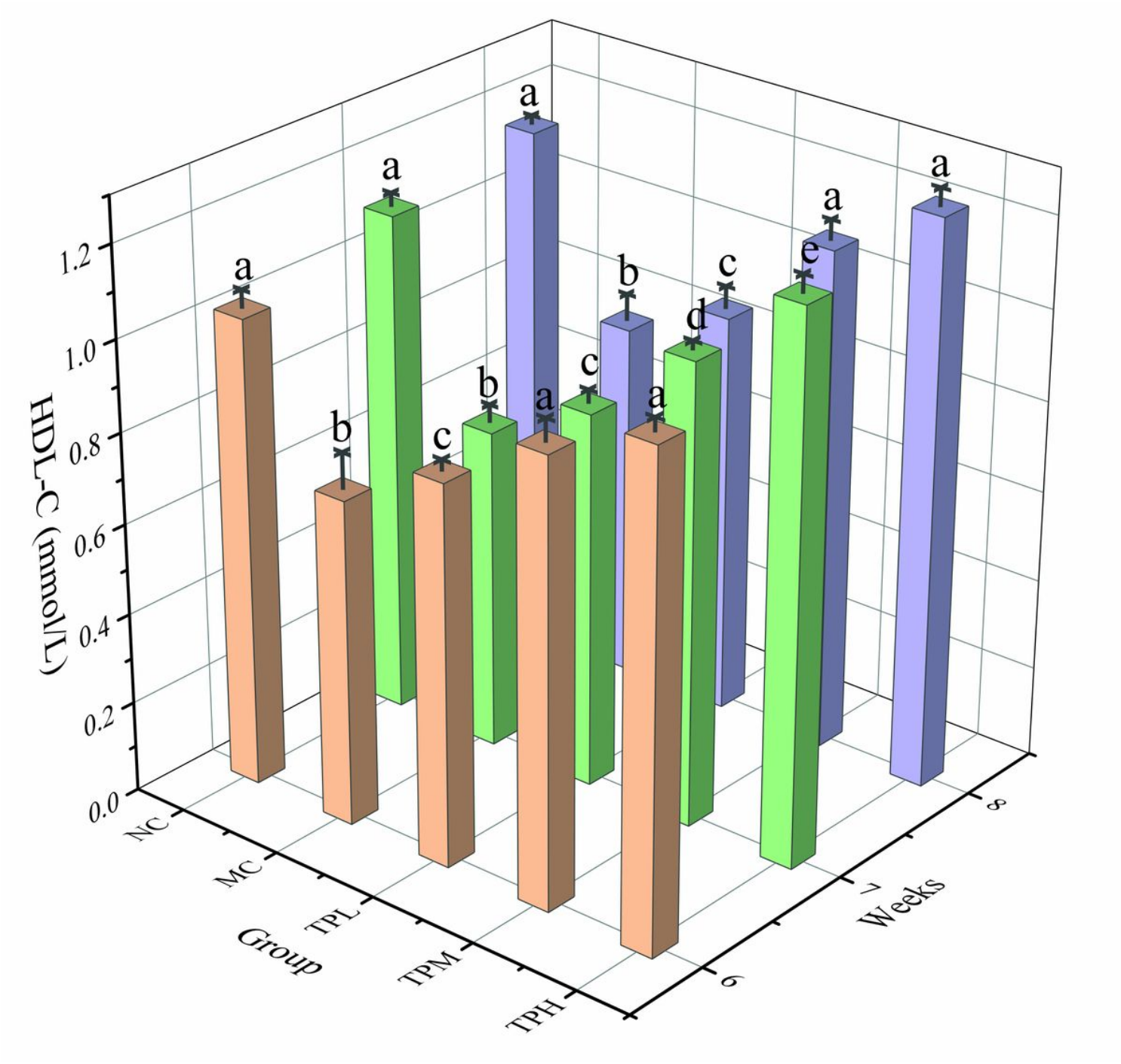


Figure 10

Changes of HDL-C content in the serum of rats fed a high-fat diet in the presence of caffeoylquinic acids obtained from a cell culture of Saussurea involucrata for 6, 7, and 8 weeks Note: In the same week, the same word means P value > 0.05, the different word means P value < 0.05.

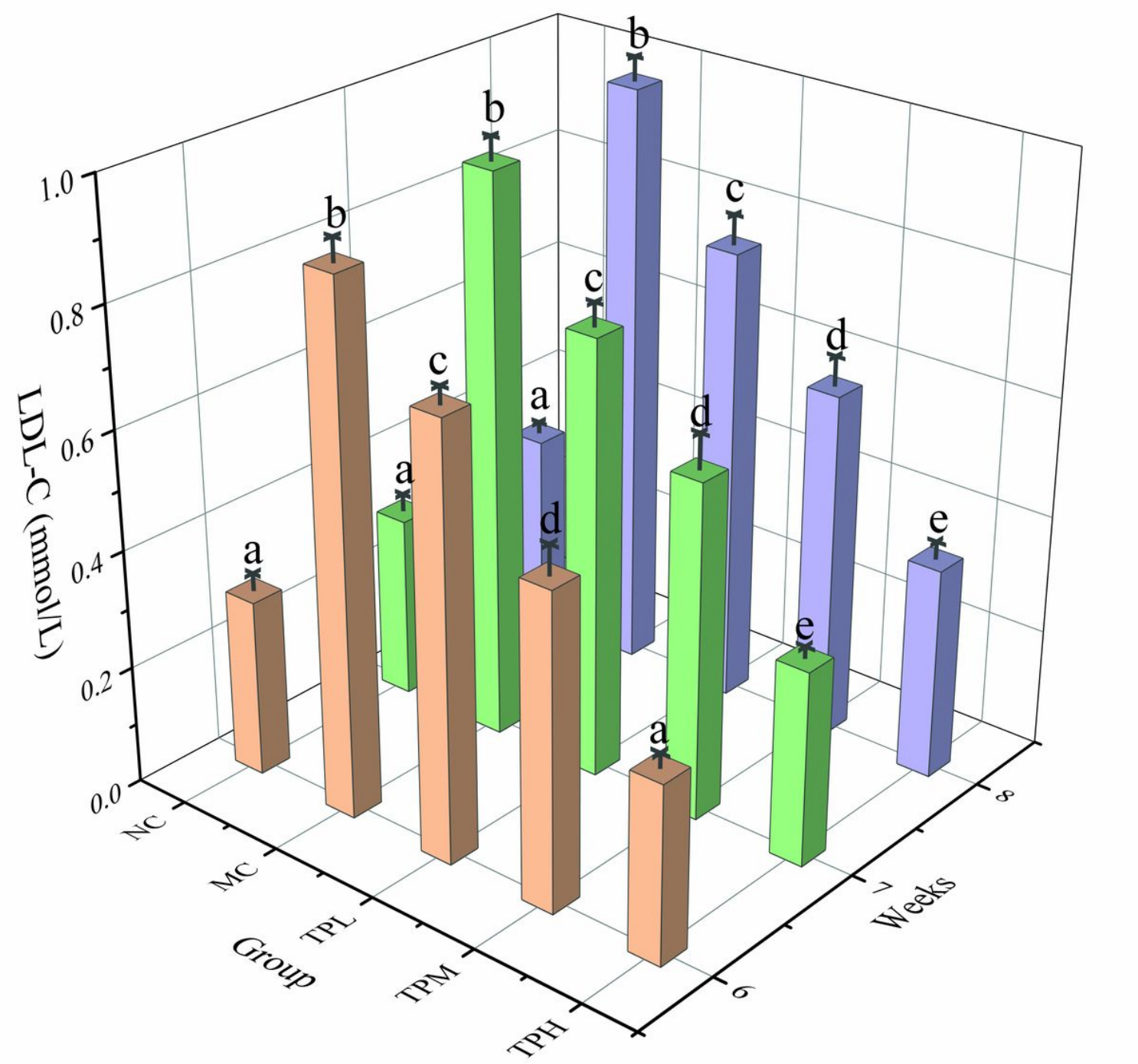


Figure 11

Changes of LDL-C content in the serum of rats fed a high-fat diet in the presence of caffeoylquinic acids obtained from a cell culture of Saussurea involucrata for 6, 7, and 8 weeks Note: In the same week, the same word means P value > 0.05, the different word means P value < 0.05.

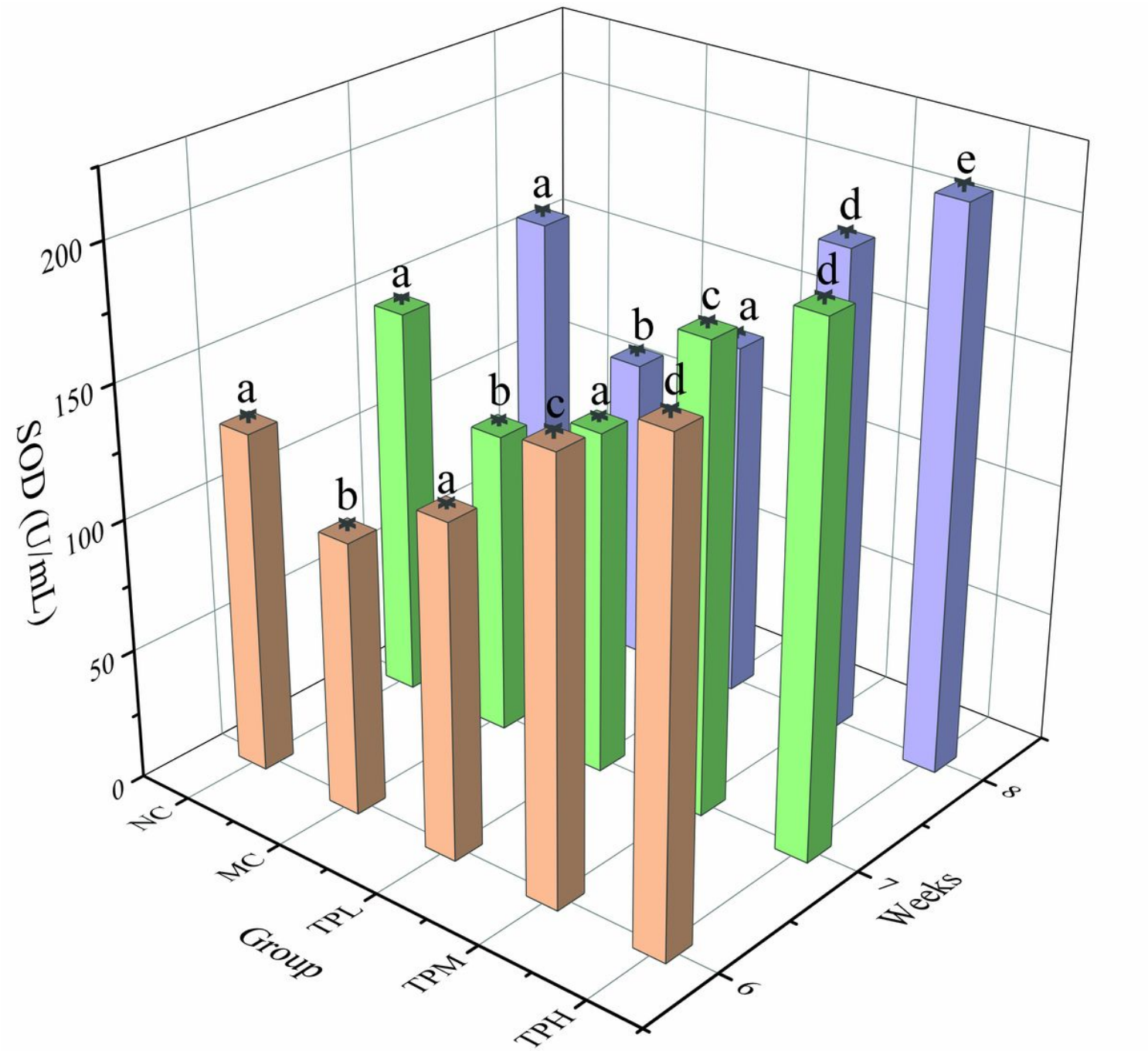


Figure 12

Changes of SOD content in the serum of rats fed a high-fat diet in the presence of caffeoylquinic acids obtained from a cell culture of Saussurea involucrata for 6, 7, and 8 weeks Note: In the same week, the same word means P value > 0.05, the different word means P value < 0.05.

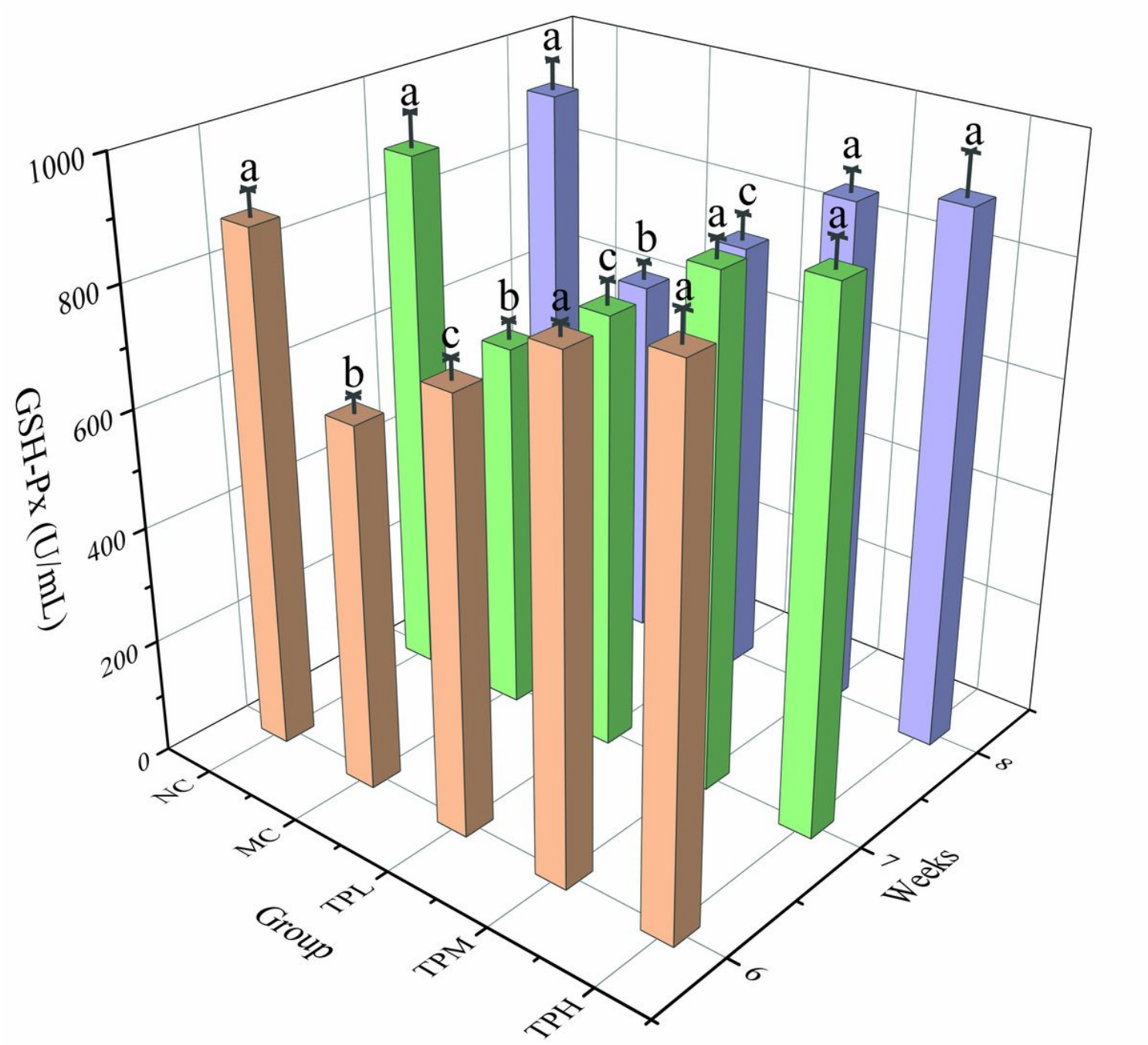


Figure 13

Changes of GSH-Px content in the serum of rats fed a high-fat diet in the presence of caffeoylquinic acids obtained from a cell culture of Saussurea involucrata for 6, 7, and 8 weeks Note: In the same week, the same word means P value > 0.05, the different word means P value < 0.05.

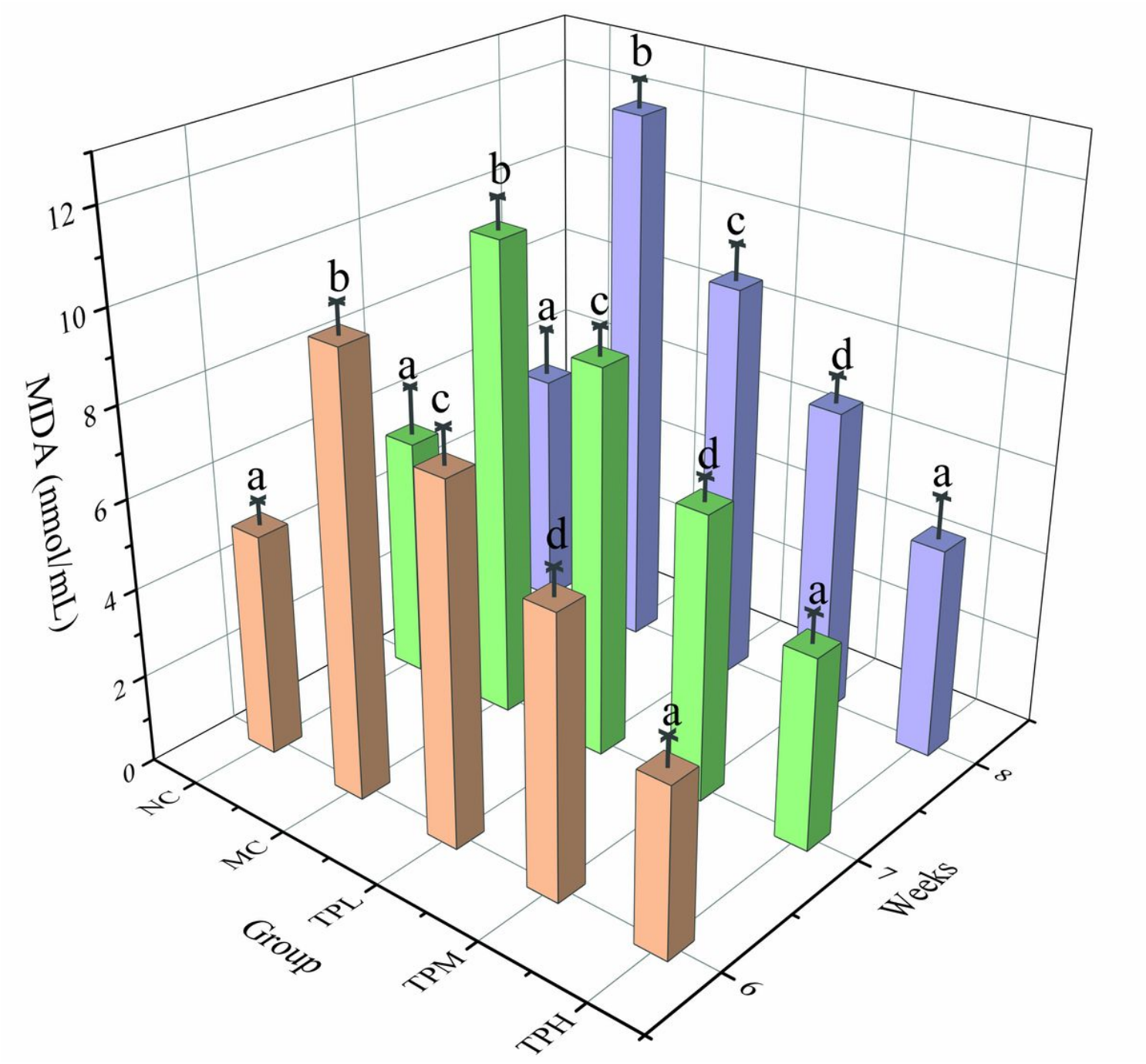


Figure 14

Changes of MDA content in the serum of rats fed a high-fat diet in the presence of caffeoylquinic acids obtained from a cell culture of Saussurea involucrata for 6, 7, and 8 weeks Note: In the same week, the same word means P value > 0.05, the different word means P value < 0.05.

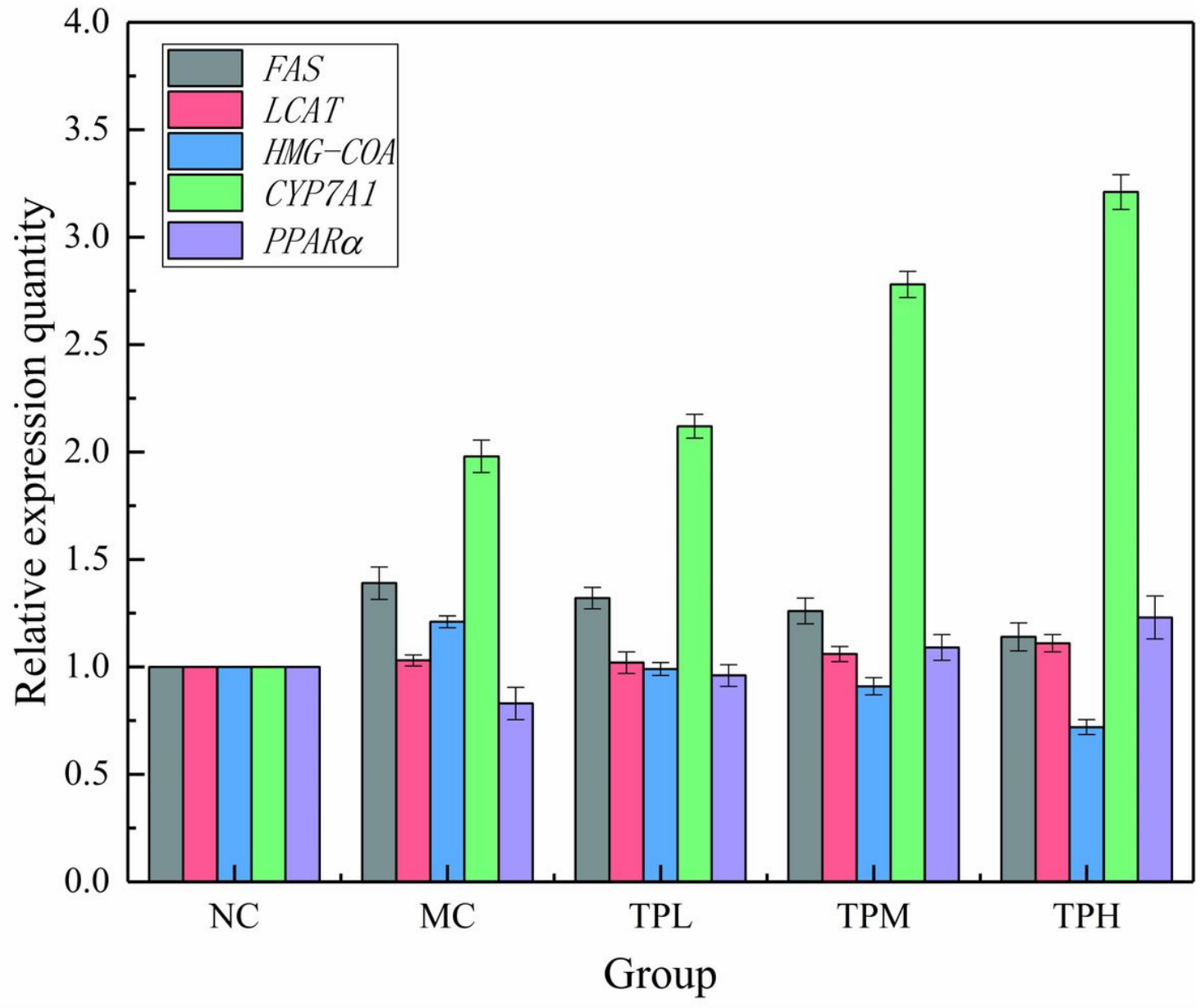


Figure 15

Gene expression levels of lipid metabolism enzymes in the liver of rats fed a high-fat diet in the presence of caffeoylquinic acids after 8 weeks of treatment

Supplementary Files

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

- Table.pdf
- SupplementaryMaterial.pdf