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Purified dietary red and white meat proteins show beneficial effects on growth and metabolism of young rats compared to casein and soy protein

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Abstract

This study compared the effects of casein, soy protein (SP), red (RMP) and white meat (WMP) proteins on growth and metabolism of young rats. Compared to casein, the ratio of daily feed intake to daily body weight gain of rats was not changed by meat protein but reduced by SP by 93.3% (P<0.05). Feeding RMP and WMP reduced the liver total cholesterol (TC) contents by 24.3% and 17.8% respectively (P<0.05). Only RMP increased plasma HDL-cholesterol concentrations (by 12.7%, P<0.05), whereas SP increased plasma triacylglycerol, TC and LDL-cholesterol concentrations by 23.7%, 19.5% and 61.5% respectively (P<0.05). Plasma essential and total amino acid concentrations were increased by WMP (by 18.8% and 12.4%, P<0.05) but reduced by SP (by 28.3 and 37.7%, P<0.05). Twenty five liver proteins were differentially expressed in response to different protein sources. Therefore, meat proteins were beneficial for growth and metabolism of young rats compared to casein and SP.

Keywords: red meat; white meat; protein quality; molecular nutrition; proteomics;

Introduction

Meat is a nutrient dense food which contains high quality protein and important micronutrients such as vitamin B12, iron and zinc[1]. Mammalian muscle meat such as beef and pork are regarded as red meat[2], whereas chicken and fish[3] are regarded as white meat. Recently, some epidemiologic studies associated high consumption of red or processed meat with several types of cancer[2]. In October, 2015, WHO released a report, which classified red and processed meat as "probably carcinogenic to humans" (Group 2A) and "carcinogenic to humans" (Group 1), respectively[2]. The publication of the report soon aroused widespread concerns about meat food all over the world. It also sparked heated debate in both academic and meat industrial areas, because the report was produced only based on the review of epidemiologic studies[4]. The reported carcinogenic effects of red and processed meat were mainly attributed to heme iron and the carcinogenic chemicals, such as N-nitroso-compounds and polycyclic aromatic hydrocarbons, that can be formed during meat processing and cooking[2]. However, it is unequivocal that lean meat is an important protein source in human diets. It has been acknowledged that meat protein has high biological availability due to its high digestibility and containing all nutritionally essential amino acids (AAs), compared to plant protein[1]. Therefore, moderate intake of meat is advised, instead of avoiding meat food.

Under the globally increasing prevalence of obesity and metabolic syndrome in both adult and children[5-6], dietary protein is regarded as the most promising macronutrient for improving of body composition and metabolic profile due to its pronounced satiating, thermogenic and lean body mass preserving effects compared to other macronutrients lipid and carbohydrate[7-9]. Until now, most of the studies on dietary protein have focused on dietary protein levels[7-10]. However, very few studies forced

on different protein sources. Milk and meat are important animal protein sources whereas soy is an important plant protein source for human health. Considering their profound differences in AA and protein compositions[1, 11-12], different biological effects were thus anticipated. Our previous study found that soy and meat proteins induce distinct physiological and metabolic responses in rats after a short time intervention (7 days)[13-15]. It has been acknowledged that the nutritional conditions in early life can profoundly influence human long-term health[16]. It was recommended by the 2015-2020 Dietary Guidelines for Americans that for children aged 2 and over, a health eating pattern should include a variety of protein foods in nutrient-dense forms from both animal and plant sources, like dairy, seafood, poultry, nuts and soy products, but reduce consumption of red meat and processed meat products[17]. These guidelines were put forward on the basis of evidence from mostly epidemiologic studies, which have shown that reduced intake of red meat as well as processed meat are associated with reduced risk of cardiovascular disease, obesity, type 2 diabetes, and some types of cancer[17]. However, there is still lack of sufficient and rigorous animal experiments to compare red meat with other protein sources. The aim of this study was to compare the effects of purified dietary protein sources from red meat, white meat, milk, and soy provided for a longer time (14 days) on growth and metabolism of young rats. To this end, young weaning rats were fed for 14 days the nutritionally balanced semi-synthetic AIN-93G diets with the only differences in protein sources. Growth, body compositions and blood biochemistry profiles were measured. To explore the molecular mechanism that may underlie the changes, liver metabolism in response to different dietary proteins were measured using 2-dimensional gel electrophoresis (2-DE) and mass spectrometry. There are three points to make our study unique. Firstly, to avoid the disturbance of the carcinogenic compounds that may be formed

during meat processing (such as curing, smoking, high cooking temperature), the purified meat protein sources were isolated from the cooked meat that was boiled in a 72°C water bath until the internal temperature reaching 70°C. Secondly, to avoid the disturbance from protein level or other nutrients, all diets in our study were prepared having the same balanced nutritional levels with the only differences in protein sources. Especially, the effects of red and white meat proteins were compared in this study. Our study provided novel evidence and important suggestions for the health effects of different protein sources in children diets.

Materials and Methods

Chemicals

Longissimus dorsi muscle of pigs and cattle and breast muscle of chicken were purchased from Su Shi Company (Nanjing, China). Dorsal muscle of fish were purchased from the local market. Diet ingredients including casein, cornstarch, dyetros, sucrose, soybean oil, cellulose, mineral mix, vitamin mix, L-Cystine and choline bitartrate were from Dyets Inc. (Bethlehem, PA). Food grade soy protein isolates were from Linyi Shansong biological products company (Linyi, China). Tissue triacylglycerol (TAG) and total cholesterol (TC) contents assay kits were from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Plasma insulin Radioimmunoassay kit were from Beijing North Institute of Biological Technology (Beijing, China). Protease inhibitor cocktail was from Roche Applied Science (Penzberg, Germany). Chemicals used for 2-dimensional gel electrophoresis including RC DC protein assay kit II, ReadyPrep 2-D cleanup kit, bio-lyte 3/10 ampholyte 40%, IPG ReadyStrip/pH3-10/11cm/12, 12% precast gels, XT MOPS running buffer, iodoacetamide were from Bio-Rad (Hercules, CA, USA). The following reagents: Tris–HCl, SDS, urea, thiourea, 3-[(3-cholamidopropyl) dimethyl

ammonio]-1-propane-sulfonate (CHAPS) and DTT were purchased from Sigma (St. Louis, MO, USA).

Animals and experimental diets

All animals were handled in accordance with the guidelines for care and use of laboratory animals of the Jiangsu Provincial Academy of Agricultural Sciences (The license number was SCXK (Su) 2002-0029). Male *Sprague Dawley* rats at 3 weeks of age were randomly assigned to 6 groups of 10 rats each. The rats had free access to water and feed through the feeding period. After one-week acclimation, the rats were fed 14 days of one of the six experimental diets that were different only in protein sources (i.e. casein, soy, chicken, fish, beef or pork). The protein sources and diets used in this study were the same with our previous study[13]. Briefly, raw meat materials were cooked in a 72°C water bath to an internal temperature of 70°C. Cooked meat were then freeze-dried and twice defatted with methylene chloride/methanol (2:1, v:v). The residual solvent was removed by evaporation and the resulting protein powder was passed through a 30 Mesh (0.595 mm) sieve. The final protein powders consisted of more than 90% of protein and 6-9% of water. All the diets were prepared according to the recommendations of the nutritionally balanced semisynthetic AIN-93G diet[18], which contained energy 4056 Kcal/Kg, protein 177 g/Kg, fat 70 g/Kg and carbohydrate 68 g/Kg. See Table 1 for specific diet formulations. To compare red and white meat proteins with casein and soy protein, beef and pork protein groups were combined as single red meat protein group (n=20), whereas chicken and fish protein groups were combined as single white meat protein group (n=20). Therefore, there were finally 4 groups of red meat protein group (n=20), white meat protein group (n=20), casein (n=10), and soy protein group (n=10).

Sample collection

During the 14 days' feeding period, body weights and dietary intakes were measured every 2 days. On the day of sacrifice, rats were deprived of feed for 4 h prior to sacrifice but were given free access to water. Rats were anaesthetized with ether inhalation. Blood was taken by orbital puncture and plasma was isolated. Liver and epididymal adipose tissues were obtained, weighed and snap frozen in liquid nitrogen. All samples were stored at -80 ^{o}C until analysis.

Liver lipid contents and plasma parameters detection

Triacylglycerol (TAG) and total cholesterol (TC) contents in the liver were determined using commercial kits purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Plasma TAG, TC, high density lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C), glucose, alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea and total protein (TP) concentrations were analyzed using a Hitachi 7180 auto analyzer (Tokyo, Japan). Plasma insulin concentrations were determined using a radioimmunoassay kit purchased from Beijing North Institute of Biological Technology (Beijing, China). The HOMA-IR[19] was calculated according to the equation IR = (fasting insulin in mU/L × fasting glucose in mM)/22.5. Plasma free AA concentrations were determined using a Hitachi L-8900 AA analyzer (Tokyo, Japan).

Two-dimensional gel electrophoresis

Protein extraction and purification. Protein extraction was performed as reported[20] with some modifications. Livers were weighed and 100 mg tissue was homogenized with 1 ml lysis buffer: 7 M urea, 2 M thiourea, 4% 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate (CHAPS, wt/vol), 65 mM DTT, 2% biolyte pH 3-10, and 1% protease inhibitor cocktail (Roche Applied Science, Penzberg, Germany). Then the sample was centrifuged at 15,000 × g for 30 min at 4 ^{o}C and the

supernatant was transfer into new tubes. Protein extract was purified using the trichloroacetic acid (TCA)/acetone precipitation method described by Li et al.[21]. Briefly, protein was precipitated in 9 volumes of 10% TCA/80% acetone solution at -20 °C for 2 h. After centrifugation at 10,000 g for 30 min at 4 °C, the supernatant was discarded and the pellet was resuspended in a rehydration buffer (7 M urea, 2 M thiourea, 1% DTT). The protein contents were determined using RC DC Protein Assay Kit (BioRad, Cat. 500-0122).

2-D gel electrophoresis. The 2-D gel was run as reported previously[21] with some modifications. Firstly, the purified protein samples were mixed with rehydration buffer (7 M urea, 2 M thiourea, 2% CHAPS (wt/vol), 1% DTT (wt/vol), 0.2% biolyte pH 3-10 (vol/vol), 0.002% bromophenol blue(wt/vol) to a final concentration of 1 mg/mL. Two hundred micrograms of protein (200 μL) was loaded on linear immobilized pH gradient strips (isoelectric point (pI) 3-10, 11 cm, BioRad, Cat. 1632014, Hercules, CA). After rehydrating at 17 ^{o}C for 12 h, isoelectric focusing was performed according to the program: 250 V (15 min), 8000 V (2.5 h) and 8000 V (35000 Vh). After finishing isoelectric focusing, the strip was first equilibrated in 5 ml equilibration buffer I (50 mM Tris–HCl, pH 8.8, 6 M urea, 20% glycerol (vol/vol), 2% SDS (wt/vol) and 1% DTT (wt/vol)) for 15 min, and then transferred to 5 ml equilibration buffer II (50 mM Tris-HCl, pH 8.8, 6 M urea, 20% glycerol (vol/vol), 2% SDS (wt/vol) and 4% (wt/vol) iodoacetamide) for 15 min. The equilibrated strip was placed on the top of a SDS-PAGE gel (12%), and then the second dimension electrophoresis was run at 200 V for 2 h at 4 ^{o}C. The 2-DE map was visualized by commassie blue staining.

Image analysis. Commassie blue stained gels were scanned, and the spots were detected and quantified with PDQuest v8.0.1 software (BioRad, Hercules, CA)

according to the software tutorial and the descriptions in other papers[22-23]. For spot identifying and gel matching, both automatic and manual editing were performed to improve accuracy. The expression level of protein spot was normalized as a percentage of the total volume of all of the spots in the gel. Statistical analysis were based on the intensities of protein spots in gels (Supplementary Table 2), while protein expression changes were represented as fold changes. The numbers of biological repetitions of 2-DE analysis of casein, soy and red meat and white protein groups were 5, 5, 10 and 10, respectively.

In-gel trypsin digestion of protein. The spots of interest were cut from the polyacrylamide gels and were destained with 500 μl of a solution (25 mM NH_3HCO_3 in 50% ACN) for 3×60 min, and then they were dehydrated using 100% ACN, reduced with 10 mM DTT at 56 ^oC, and alkylated with 55 mM iodoacetamide without light exposure. Afterwards the samples were treated with 50 μl trypsin solution (1 μg trypsin in 100 μl 25 mM ammonium hydrogen carbonate in 25% ACN, pH 8.0) at 37 °C overnight.

Protein identification by mass spectrometry and functional analysis. Proteins were identified by MALDI-TOF/TOF. The MS/MS data were searched against Mascot 2.3.02 (Matrix Science) applied to NCBI Rattus 1031(51807 seqs) based on the following search parameters: peptide mass tolerance: 100ppm; fragment mass tolerance, 0.6 Da; fixed modifications: Carbamidomethyl (C); variable modifications: Gln->pyro-Glu (N-term Q), Oxidation (M) and Deamidated (NQ); max missed cleavages: one. Significant scores > 70 and at least five peptide matches for each protein were used as criteria for positive protein identification. The gene ontology (GO) interpretation of proteins was done using PANTHER analysis[24].

Statistical methods

The diet effect on measured variables were analyzed by one-way ANOVA and means were compared by least significant difference (LSD) multiple comparison. Statistical significance was set at $P < 0.05$. Values are shown as means ± SD.

Results

Body weight and body adiposity

Rats in red or white meat protein groups had slightly higher initial body weights (IBWs) than the rats in casein group ($P < 0.05$, Figure 1A), whereas the IBWs of the rats in soy protein group were not different from casein or meat protein groups. Feeding red or white meat protein diets significantly increased the daily feed intakes (DFIs), daily body weight gains (DBWGs) and final body weights (FBWs) of rats. However, the DFI/DBWG ratio was not different between meat proteins and casein groups (Figure 1E). Feeding soy protein diet significantly reduced DBWGs (by 47.7%) and FBWs (by 22.7%) of rats ($P < 0.05$, Figure 1B) without affecting the DFIs compared to casein. As a result, the DFI/DBWG ratio was significantly increased by dietary soy protein compared to casein ($P < 0.05$, Figure 1E).

In order to evaluate the effects of different dietary protein sources on body adiposity, epididymal adipose tissue weight (EATW) and liver lipid contents were measured (Figure 2). Compared to casein, the percentage of EATW to BW was not affected by meat or soy proteins ($P > 0.05$, Figure 2A2). When compared between meat proteins and soy protein, the percentage of EATW to BW was lower for the soy protein group than meat protein groups. Liver TC contents were significantly reduced by red (by 24.3%, $P < 0.05$) or white meat proteins (by 17.8%, $P < 0.05$) but were not affected by soy protein compared to casein. The changes in liver TAG contents did not reach the significant level. Liver weight was reduced by soy, red meat and white meat proteins compared to casein ($P < 0.05$, Figure 2B).

Plasma profiles

Plasma lipid concentrations were significantly changed by different dietary protein sources (Figure 3). Plasma TAG concentrations were significantly increased by soy protein intake (by 23.7%, $P < 0.05$) but were not affected by red or white meat proteins compared to casein (Figure 3A1). When compared between red meat and white meat proteins, the rats fed white meat protein had lower plasma TAG concentration than the rats fed red meat protein (Figure 3A1). The pattern of the plasma TC concentration changes was the same with the plasma TAG concentrations regulated by dietary casein, soy, and meat proteins (Figure 3A2). Only red meat proteins increased the plasma HDL-C concentrations (Figure 3A3, by 12.7%, $P < 0.05$) in rats. Only soy protein increased the plasma LDL-C concentrations in rats (Figure 3A4, by 61.5%, $P < 0.05$). Plasma glucose concentrations, insulin level and HOMA-IR were significantly reduced by soy protein ($P < 0.05$, Figure 3B). Only red meat protein increased the plasma insulin levels and HOMA-IR.

Because that liver weights of rats were reduced by dietary soy and meat proteins, therefore plasma biomarkers for liver health, i.e. AST and ALT[25], were measured. The ratio of AST to ALT was calculated (Figure 4A). It was showed that plasma AST and ALT concentrations were significantly increased by soy protein (increased by 74.8% and 86.8%, respectively, $P < 0.05$) and white meat protein (increased by 26.2% and 34.2%, respectively, $P < 0.05$) but were not changed by red meat protein compared to casein (Figure 4A1 & A2). Notably, no significant changes were observed in the ratio of AST to ALT in any group (Figure 4A3). Plasma urea and total protein concentrations were measured to indicate the changes of AA degradation[26] and protein synthesis[27] in the liver. Only soy protein increased plasma urea concentrations (increased by 32%, $P < 0.05$, Figure 4B2) but reduced plasma total protein

241 concentrations (reduced by 6.8%, $P < 0.05$, Figure 4B1). At the same time, plasma
242 total AA concentrations were significantly reduced by soy protein compared to casein
243 (reduced by 28.3%, $P < 0.05$, Table 2), among which the essential AA concentrations
244 were reduced by 37.7% ($P < 0.05$) and non-essential AA concentrations were reduced
245 by 16.3% ($P < 0.05$). In contrast, feeding white meat protein increased plasma
246 essential and total AA concentrations compared to casein (increased by 18.8% and
247 12.4%, respectively, $P < 0.05$), whereas feeding red meat protein to rats did not affect
248 their plasma essential and total AA concentrations.

249 **Liver protein expression changes**

250 The liver protein expressions were evaluated using 2-DE. Twenty five proteins were
251 identified as differentially expressed in response to different dietary protein sources
252 (Table 3). One liver protein relating to ATP biosynthesis (Atp5a1, ATP synthase
253 subunit alpha) was significantly upregulated by dietary soy, white meat and red meat
254 proteins compared to casein. Several proteins involving in AA metabolism, such as
255 GOT1 (aspartate aminotransferase, AST), OTC (ornithine carbamoyltransferase, urea
256 cycle), ALDH6A1 (methylmalonate-semialdehyde dehydrogenase, valine metabolic
257 process) and MAT1A (s-adenosylmethionine synthase isoform type-1, methionine
258 metabolic process), protein biosynthesis (EF1A1, elongation factor 1-alpha 1) and
259 gluconeogenesis (FBP1, fructose-1,6-bisphosphatase 1) were significantly
260 upregulated by dietary soy protein only ($P < 0.05$). On the contrary, several proteins
261 relating to proteolysis (LAP3, cytosol aminopeptidase), protein transport (GCC2,
262 GRIP and coiled-coil domain-containing protein 2), glycolysis (PKLR, Pyruvate
263 kinase PKLR), and triacylglycerol biosynthesis (GPD1, Glycerol-3-phosphate
264 dehydrogenase [NAD(+)]) were significantly downregulated by dietary soy protein
265 only. Two liver proteins relating to iron ion transport (TF, serotransferrin) and

response to oxidative stress (PRDX1, Peroxiredoxin-1) were upregulated by soy and white meat proteins. In addition, seven liver proteins were found upregulated specifically by dietary white meat protein, among which four proteins were dehydrogenases and five proteins were in mitochondrion. These proteins were mainly related to oxidation reactions in mitochondrion including processes of fatty acid oxidation and electron transport. Two liver proteins relating to lactate metabolic process (LDHA, L-lactate dehydrogenase A chain) and glycolysis (PKLR, pyruvate kinase PKLR) were upregulated only by dietary red meat protein. Two other liver proteins relating to hydrogen peroxide catabolic process (CAT, catalase) and tricarboxylic acid cycle (MDH2, malate dehydrogenase) were upregulated and one liver protein relating to transsulfuration (MPST, 3-mercaptopyruvate sulfurtransferase) was downregulated by both dietary white and red meat proteins.

Discussion

This study compared the effects of dietary purified protein sources from milk, red meat, white meat and soy provided at the nutritional recommended level on growth, body compositions, blood insulin, lipid and AA profiles and live protein expression in young weaning rats. Casein was chosen as reference protein source because from a nutritional perspective it is a high-quality protein, and it is therefore used as protein source in the well-balanced semi-synthetic AIN-93G diet[18]. The AIN-93 diet is the global standard for a purified rodent diets proposed by the American Institute of Nutrition (AIN), and is considered as ‘golden standard’ in nutrition research. We therefore used the AIN-93G diet as reference diet. For nutritional studies of protein/amino acids, laboratory rats have been recommended and are generally accepted as a valid animal model for predicting protein/amino acid nutrition and metabolism in humans[28-29]. Most of the early work about dietary amino acid tolerance

was done with rats fed casein-based purified diets[30]. It has been suggested that use of diets containing mixed ingredients and with normal protein levels is probably more relevant in terms of extrapolation to humans[30]. In our study, we used rats as animal model, and the casein-based semi-synthetic diet (AIN-93G) was used as the reference diet. All diets used in our study have normal protein levels but different protein sources. Therefore, we believe the findings in our study might be relevant to humans. Except for rodent, the farm animals like pigs have also been commonly used in protein/amino acid studies[28-29]. Recently, the voice of promoting the use of pigs as animal model for human nutrition study is increasing[31-32]. However, the early studies with pigs (farm animals) were usually oriented to the immediate objective of improving food production. This is quite different from human nutrition, in which costs and efficiency of nutrient usage are often not overriding concerns[28]. Therefore, compared to studies with rats, the results from studies with pigs are less comparable to human nutrition.

Our results showed that compared to meat proteins, feeding soy protein diet significantly reduced the DFI of the rats, which was independent of the IBW of the rats. These results were consistent with our previous study[13], in which the rats were fed the same diets for a shorter time (7 days). As proved in our previous study, the feed intake inhibition effects of dietary soy protein to the young rats were attributed to the AA limitation (methionine) in the soy protein source. This was also found in the present study from the responses of plasma AA concentrations in young rats. In the present study the plasma total AA concentrations in the young rats fed soy protein diet were significantly reduced (by 28.3%), among which the essential AA concentrations were especially reduced (by 37.7 %). Notably, plasma methionine and valine concentrations was significantly reduced by more than 40% by dietary soy protein.

316 This was correlated to the liver proteins expression relating to methionine and valine
317 metabolisms that were significantly upregulated by dietary soy protein only. On the
318 contrary, white meat protein intake increased both essential and total AA
319 concentrations in rats' plasma, while dietary red meat had similar effects with casein
320 on plasma total AA concentrations. It has been proved that elevated intake of dietary
321 protein can regulate feed intake due to high satiety[7-9, 33]. The study from Hall et al
322 (2003) showed that whey protein increased the satiety in human subjects compared to
323 casein[34], indicating that satiety can be regulated by different protein sources. However,
324 previous studies showed that under the condition of dietary AA limitation, the meal
325 termination is not due to satiety, which was evidenced by the absence of the satiety
326 sequence[35-36]. The underlying mechanisms of the feed intake depression effects of
327 dietary AA limitation have been well reviewed[35]. Therefore, we concluded that the
328 feed intake reduction effects of the dietary soy protein was caused by the AA
329 limitation but not by satiety that may affected by dietary soy protein. It is also
330 suggested that when study the effects of different protein sources on satiety, the AA
331 compositions of protein sources should be considered firstly.
332 In order to evaluate the effects of different protein sources on growth of young rats,
333 the ratio of DFI/DBWG were calculated. Both white and red meat proteins had similar
334 DFI/DBWG ratios with casein indicating that meat proteins had similar effects with
335 milk protein on regulation of growth of young rats. However, compared to casein and
336 meat proteins, dietary soy protein had a significantly higher DFI/DBWG ratio. This
337 indicated that when feeding the same amount of soy protein, casein or meat proteins,
338 the body weight gain of the young rats fed soy protein will be much lower (by about
339 50%) than the rats fed casein or meat proteins. The body compositions of the young
340 rats after 14 days' consumption of different protein diets were measured. It was found

that the adipose tissue mass and liver weight of rats were significantly reduced by dietary soy protein. At the same time, the negative body nitrogen and protein balances were observed in the rats fed soy protein diet according to the changes in plasma urea and total protein concentrations, which are biomarkers for body nitrogen and total protein balance[26]. It was showed that plasma urea concentration were significantly increased but plasma total protein concentration were significantly reduced by dietary soy protein intake. Unlike soy protein, plasma urea and total protein concentrations were similar between casein, red meat and white meat protein groups. This indicates that meat proteins are more balanced protein sources than soy protein in term of body protein metabolism. The liver plays an important role in regulating AA and protein metabolism. Since in the present study the liver weights of young rats were significantly reduced by both dietary soy and meat proteins compared to casein. In order to evaluate the health status of the liver, plasma AST/ALT ratio was calculated[25]. It was showed that no significant changes were observed in AST/ALT ratios, indicating that the liver function was not impaired by any dietary protein sources in this study. Only the individual plasma AST or ALT concentrations were increased by dietary soy and white meat proteins. This was consistent with the changes in liver protein expression of GOT1 (i.e. AST), which was significantly upregulated by dietary soy protein only. The increased AST and ALT indicated that the AA metabolism in the liver was activated by soy protein and white meat protein. However, the mechanisms are different between soy and white meat protein. For soy protein, this was caused by AA limitation (low plasma AA concentrations)) and will lead to negative nitrogen balance. For white meat protein, this was caused by AA excess (high plasma AA concentrations) and will lead to AA waste. Although, the plasma total protein concentrations was reduced specifically by dietary soy protein, the liver

protein expression relating to protein biosynthesis was increased but the liver protein expression relating to proteolysis was reduced specifically by dietary soy protein. This was suggested to be a compensatory increase in protein synthesis in response to inadequate in essential AA intake in soy protein group.

Accordingly, not just for adult people, cardiovascular morbidity can now be considered to be, in part, a prenatal and pediatric disease[16]. Blood TG, TC, HDL-C and LDL-C are important biomarkers for lipid homeostasis and thus the cardiovascular diseases. It has been found that soy protein may have beneficial effects on lipid metabolism. However, in this study we found that soy protein had deleterious effects on liver adiposity and blood lipid profiles, whereas both red and white meat proteins showed beneficial effects. Specifically, dietary red and white meat proteins reduced the liver TC contents. Feeding red meat protein increased the plasma HDL-C concentration. When analyzing metabolism in the liver, we found that feeding white meat protein diets increased fatty acid beta-oxidation. Whereas dietary soy protein had no significant effects on liver lipid contents but increased the plasma TAG, TC and LDL-C concentrations.

Insulin resistance is the main mechanism for type 2 diabetes and a main component for metabolic syndrome. Notably, plasma insulin and HOMA-IR levels were significantly higher in the rats fed red meat protein than white meat protein, casein and soy protein groups. This suggest that red meat may increase the risk of type 2 diabetes (T2D). Findings from epidemiologic studies also suggest positive associations of red meat with risk of T2D[37]. However, it is unclear whether it is the protein per se or other components of protein-rich foods in those epidemiologic studies. Energy metabolism in the liver were significantly increased by white meat protein compared to red meat protein. This can be related to the increased blood AA

391 concentrations after intake of white meat protein. This was supported by other study
392 that rapid increase of AA concentrations after a meal is related to stimulation of
393 oxidation and protein syntheses[38]. The study from Mikkelsen et al (2000)[39] found
394 animal protein in pork meat produced a 2% higher 24-h energy expenditure than did
395 the vegetable protein in soy.
396 Notably, our 2-DE analysis results showed that iron transport protein serotransferrin
397 (short name: transferrin) was significantly upregulated in the liver of rats fed soy
398 protein and white meat protein diets compared to casein and red meat protein groups.
399 This indicated that dietary soy or white meat protein intake increased liver transferrin
400 synthesis. Transferrin is mainly synthesized in the liver[40]. The main role of transferrin
401 is to transport iron from sites of absorption (duodenum) and red blood cell recycling
402 (macrophages) to tissues for storage (liver) and utilization (bone marrow)[40-41]. A high
403 transferrin level may indicate iron deficiency which is often seen in patients suffering
404 from iron deficiency anemia[40] and also in the rats fed a low-iron diet[42]. Therefore, we
405 deduced that the increased liver transferrin level found in the rats fed soy and white
406 meat protein diets in our study can be attributed to the null heme iron (highly
407 bioavailable iron) in the soy protein source and relative low heme iron contents in the
408 white meat protein sources compared to red meat protein sources[43]. Except for the
409 differences in iron content directly, it has been proved that dietary protein can also
410 affect iron absorption[44-45]. Etcheverry et al (2006) assessed the effects of beef and soy
411 proteins on the bioavailability of non-heme iron in children. Their findings indicated
412 that beef protein increased non-heme iron absorption compared to soy protein[46]. Iron
413 deficiency remain substantial problems in small children in both developed and
414 developing nations[47]. Therefore, when designing diets for children, the effect of
415 protein source on iron absorption should be one of the factors taken into account.

Taken together, dietary soy protein showed deleterious effects on liver adiposity and blood lipid profiles and induced negative nitrogen balance and growth inhibition in young rats due to its limitation in essential AAs. In contrast to soy protein, both red and white meat proteins showed beneficial effects on growth and lipid metabolism of rats. Thus, soy protein is not an optimal protein source for growth and metabolism health of young animals, while meat protein is if not better than but at least as well as milk protein to the growth and metabolism health of young animals.

There were still some limitations in this study. The treatment time was 14 days, which was a single time point and relatively short. To better understand the process and the development of metabolism changes, longer feeding time or different time points could be studied and compared in future studies. The age of the rats could affect some parts of the responses to dietary proteins. Since we did not include rats with different ages in this study, it is difficult, if not impossible to tell which parts. The study investigates the effects of normal meat protein levels. It would be interesting to test the effects of higher levels of meat proteins on metabolism in future. Therefore, more studies are needed to get a comprehensive understanding of health effects of meat proteins and its molecular mechanisms.

Abbreviations Used

2-DE: two dimensional gel electrophoresis; AA: amino acid; DBWG: daily body weight gain; DFI: daily feed intake; DFI/DBWG: ratio of daily feed intake to daily body weight gain; EATW: absolute weight of epididymal adipose tissue; EATW/BW: relative weight of epididymal adipose tissue to body weight; FBW: final body weight; HDL-C: high density lipoprotein-cholesterol; IBW: initial body weight; LDL-C: low density lipoprotein-cholesterol; LW: absolute weight of liver; LW/BW: relative weight of liver to body weight; T2D: type 2 diabetes; TAG: triacylglycerol; TAG-L:

441 triacylglycerol in the liver; TC: total cholesterol; TCA: trichloroacetic acid; TC-L:
442 total cholesterol in the liver; TP: total protein

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447 **References**

448 1. Pereira, P. M.; Vicente, A. F., Meat nutritional composition and nutritive role in
449 the human diet. *Meat science* **2013,** *93* (3), 586-92.

450 2. Bouvard, V.; Loomis, D.; Guyton, K. Z.; Grosse, Y.; Ghissassi, F. E.;
451 Benbrahim-Tallaa, L.; Guha, N.; Mattock, H.; Straif, K.; International Agency for
452 Research on Cancer Monograph Working, G., Carcinogenicity of consumption of red
453 and processed meat. *The Lancet. Oncology* **2015**.

454 3. Kiessling, A.; Ruohonen, K.; Bjørnevik, M., Muscle fibre growth and quality in
455 fish. *Arch Tierz Dummerstorf* **2006,** *49*, 137-146.

456 4. Klurfeld, D. M., Research gaps in evaluating the relationship of meat and health.
457 *Meat science* **2015,** *109*, 86-95.

458 5. Flynn, M. A.; McNeil, D. A.; Maloff, B.; Mutasingwa, D.; Wu, M.; Ford, C.;
459 Tough, S. C., Reducing obesity and related chronic disease risk in children and youth:
460 a synthesis of evidence with 'best practice' recommendations. *Obesity reviews : an*
461 *official journal of the International Association for the Study of Obesity* **2006,** *7 Suppl*
462 *1*, 7-66.

463 6. Mellendijk, L.; Wiesmann, M.; Kiliaan, A. J., Impact of Nutrition on Cerebral
464 Circulation and Cognition in the Metabolic Syndrome. *Nutrients* **2015,** *7* (11),
465 9416-9439.

7. Westerterp-Plantenga, M. S., The significance of protein in food intake and body weight regulation. *Current opinion in clinical nutrition and metabolic care* **2003,** *6* (6), 635-8.

8. Arentson-Lantz, E.; Clairmont, S.; Paddon-Jones, D.; Tremblay, A.; Elango, R., Protein: A nutrient in focus. *Applied physiology, nutrition, and metabolism = Physiologie appliquee, nutrition et metabolisme* **2015,** *40* (8), 755-61.

9. Moore, D. R.; Soeters, P. B., The Biological Value of Protein. *Nestle Nutrition Institute workshop series* **2015,** *82*, 39-51.

10. Schwarz, J.; Tome, D.; Baars, A.; Hooiveld, G. J.; Muller, M., Dietary protein affects gene expression and prevents lipid accumulation in the liver in mice. *PloS one* **2012,** *7* (10), e47303.

11. Elango, R.; Ball, R. O.; Pencharz, P. B., Amino acid requirements in humans: with a special emphasis on the metabolic availability of amino acids. *Amino acids* **2009,** *37* (1), 19-27.

12. Wen, S.; Zhou, G.; Song, S.; Xu, X.; Voglmeir, J.; Liu, L.; Zhao, F.; Li, M.; Li, L.; Yu, X.; Bai, Y.; Li, C., Discrimination of in vitro and in vivo digestion products of meat proteins from pork, beef, chicken, and fish. *Proteomics* **2015,** *15* (21), 3688-98.

13. Song, S.; Hooiveld, G. J.; Li, M.; Zhao, F.; Zhang, W.; Xu, X.; Muller, M.; Li, C.; Zhou, G., Dietary soy and meat proteins induce distinct physiological and gene expression changes in rats. *Scientific reports* **2016,** *6*, 20036.

14. Song, S.; Hooiveld, G. J.; Wei, Z.; Li, M.; Fan, Z.; Jing, Z.; Xu, X.; Muller, M.; Li, C.; Zhou, G., Comparative Proteomics Provides Insights into Metabolic Responses in Rat Liver to Isolated Soy and Meat Proteins. *Journal of Proteome Research* **2016,** *15* (4), 1135-1142.

15. Song, S.; Hooiveld, G. J.; Li, M.; Zhao, F.; Zhang, W.; Xu, X.; Muller, M.; Li, C.;

Zhou, G., Distinct physiological, plasma amino acid, and liver transcriptome responses to purified dietary beef, chicken, fish, and pork proteins in young rats. *Molecular nutrition & food research* **2016,** *60* (5), 1199-205.

16. Hochberg, Z.; Feil, R.; Constancia, M.; Fraga, M.; Junien, C.; Carel, J. C.; Boileau, P.; Le Bouc, Y.; Deal, C. L.; Lillycrop, K.; Scharfmann, R.; Sheppard, A.; Skinner, M.; Szyf, M.; Waterland, R. A.; Waxman, D. J.; Whitelaw, E.; Ong, K.; Albertsson-Wikland, K., Child health, developmental plasticity, and epigenetic programming. *Endocrine reviews* **2011,** *32* (2), 159-224.

17. Agriculture, U. S. D. o. H. a. H. S. a. U. S. D. o., 2015-2020 Dietary Guidelines for Americans. 8th Edition ed.; **2015**.

18. Reeves, P. G.; Nielsen, F. H.; Fahey Jr, G. C., AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. *J nutr* **1993,** *123* (11), 1939-1951.

19. Cacho, J.; Sevillano, J.; de Castro, J.; Herrera, E.; Ramos, M., Validation of simple indexes to assess insulin sensitivity during pregnancy in Wistar and Sprague-Dawley rats. *American Journal of Physiology-Endocrinology and Metabolism* **2008,** *295* (5), E1269-E1276.

20. Hao, R.; Adoligbe, C.; Jiang, B.; Zhao, X.; Gui, L.; Qu, K.; Wu, S.; Zan, L., An Optimized Trichloroacetic Acid/Acetone Precipitation Method for Two-Dimensional Gel Electrophoresis Analysis of Qinchuan Cattle Longissimus Dorsi Muscle Containing High Proportion of Marbling. *PloS one* **2015,** *10* (4), e0124723.

21. Li, C. B.; Li, J.; Zhou, G. H.; Lametsch, R.; Ertbjerg, P.; Bruggemann, D. A.; Huang, H. G.; Karlsson, A. H.; Hviid, M.; Lundstrom, K., Electrical stimulation affects metabolic enzyme phosphorylation, protease activation, and meat tenderization in beef. *Journal of animal science* **2012,** *90* (5), 1638-49.

516 22. Parodi-Talice, A.; Monteiro-Goes, V.; Arrambide, N.; Avila, A. R.; Duran, R.;
517 Correa, A.; Dallagiovanna, B.; Cayota, A.; Krieger, M.; Goldenberg, S.; Robello, C.,
518 Proteomic analysis of metacyclic trypomastigotes undergoing Trypanosoma cruzi
519 metacyclogenesis. *Journal of mass spectrometry : JMS* **2007,** *42* (11), 1422-32.
520 23. Zheng, A.; Liu, G.; Zhang, Y.; Hou, S.; Chang, W.; Zhang, S.; Cai, H.; Chen, G.,
521 Proteomic analysis of liver development of lean Pekin duck (Anas platyrhynchos
522 domestica). *Journal of proteomics* **2012,** *75* (17), 5396-413.
523 24. Thomas, P. D.; Campbell, M. J.; Kejariwal, A.; Mi, H.; Karlak, B.; Daverman, R.;
524 Diemer, K.; Muruganujan, A.; Narechania, A., PANTHER: a library of protein
525 families and subfamilies indexed by function. *Genome research* **2003,** *13* (9),
526 2129-41.
527 25. Giannini, E.; Botta, F.; Fasoli, A.; Ceppa, P.; Risso, D.; Lantieri, P. B.; Celle, G.;
528 Testa, R., Progressive liver functional impairment is associated with an increase in
529 AST ALT ratio. *Digest Dis Sci* **1999,** *44* (6), 1249-1253.
530 26. Leung, P. M.; Rogers, Q. R.; Harper, A. E., Effect of amino acid imbalance on
531 plasma and tissue free amino acids in the rat. *J Nutr* **1968,** *96* (3), 303-18.
532 27. Hoffenberg, R., Measurement of the synthesis of liver-produced plasma proteins
533 with special reference to their regulation by dietary protein and amino acid supply.
534 *Proceedings of the Nutrition Society* **1972,** *31* (03), 265-272.
535 28. Bergen, W. G., Contribution of research with farm animals to protein metabolism
536 concepts: a historical perspective. *Journal of Nutrition* **2007,** *137* (3), 706.
537 29. Deglaire, A.; Moughan, P. J., Animal models for determining amino acid
538 digestibility in humans - a review. *British Journal of Nutrition* **2012,** *108 Suppl 2* (4),
539 S273.
540 30. Baker, D. H., Animal models in nutrition research. *Journal of Nutrition* **2008,** *138*

(2), 391.

31. Spurlock, M. E.; Gabler, N. K., The development of porcine models of obesity and the metabolic syndrome. *Journal of Nutrition* **2008,** *138* (2), 397.

32. Gonzalez, L. M.; Moeser, A. J.; Blikslager, A. T., Porcine models of digestive disease: the future of large animal translational research. *Translational Research the Journal of Laboratory & Clinical Medicine* **2015,** *166* (1), 12-27.

33. Westerterp-Plantenga, M. S., Protein intake and energy balance. *Regulatory peptides* **2008,** *149* (1-3), 67-9.

34. Hall, W. L.; Millward, D. J.; Long, S. J.; Morgan, L. M., Casein and whey exert different effects on plasma amino acid profiles, gastrointestinal hormone secretion and appetite. *The British journal of nutrition* **2003,** *89* (2), 239-48.

35. Gietzen, D. W.; Hao, S.; Anthony, T. G., Mechanisms of food intake repression in indispensable amino acid deficiency. *Annual review of nutrition* **2007,** *27*, 63-78.

36. Feurte, S.; Nicolaidis, S.; Berridge, K. C., Conditioned taste aversion in rats for a threonine-deficient diet: demonstration by the taste reactivity test. *Physiology & behavior* **2000,** *68* (3), 423-9.

37. Aune, D.; Ursin, G.; Veierød, M. B., Meat consumption and the risk of type 2 diabetes: a systematic review and meta-analysis of cohort studies. *Diabetologia* **2009,** *52* (11), 2277-2287.

38. Boirie, Y.; Dangin, M.; Gachon, P.; Vasson, M. P.; Maubois, J. L.; Beaufrere, B., Slow and fast dietary proteins differently modulate postprandial protein accretion. *Proceedings of the National Academy of Sciences of the United States of America* **1997,** *94* (26), 14930-5.

39. Mikkelsen, P. B.; Toubro, S.; Astrup, A., Effect of fat-reduced diets on 24-h energy expenditure: comparisons between animal protein, vegetable protein, and

566 carbohydrate. *The American journal of clinical nutrition* **2000,** *72* (5), 1135-41.

567 40. Macedo, M. F.; de Sousa, M., Transferrin and the transferrin receptor: of magic

568 bullets and other concerns. *Inflammation & allergy drug targets* **2008,** *7* (1), 41-52.

569 41. Matsuo, S.; Ogawa, M.; Muckenthaler, M. U.; Mizui, Y.; Sasaki, S.; Fujimura, T.;

570 Takizawa, M.; Ariga, N.; Ozaki, H.; Sakaguchi, M.; Gonzalez, F. J.; Inoue, Y.,

571 Hepatocyte Nuclear Factor 4alpha Controls Iron Metabolism and Regulates

572 Transferrin Receptor 2 in Mouse Liver. *The Journal of biological chemistry* **2015**.

573 42. Idzerda, R. L.; Huebers, H.; Finch, C. A.; McKnight, G. S., Rat transferrin gene

574 expression: tissue-specific regulation by iron deficiency. *Proceedings of the National*

575 *Academy of Sciences of the United States of America* **1986,** *83* (11), 3723-7.

576 43. McGuire, S., US Department of Agriculture and US Department of Health and

577 Human Services, Dietary Guidelines for Americans, 2010. Washington, DC: US

578 Government Printing Office, January 2011. *Advances in Nutrition: An International*

579 *Review Journal* **2011,** *2* (3), 293-294.

580 44. Hurrell, R.; Egli, I., Iron bioavailability and dietary reference values. *The*

581 *American journal of clinical nutrition* **2010,** *91* (5), 1461S-1467S.

582 45. Weinborn, V.; Pizarro, F.; Olivares, M.; Brito, A.; Arredondo, M.; Flores, S.;

583 Valenzuela, C., The Effect of Plant Proteins Derived from Cereals and Legumes on

584 Heme Iron Absorption. *Nutrients* **2015,** *7* (11), 8977-86.

585 46. Etcheverry, P.; Hawthorne, K. M.; Liang, L. K.; Abrams, S. A.; Griffin, I. J.,

586 Effect of beef and soy proteins on the absorption of non-heme iron and inorganic zinc

587 in children. *Journal of the American College of Nutrition* **2006,** *25* (1), 34-40.

588 47. Villalpando, S.; Cruz Vde, L.; Shamah-Levy, T.; Rebollar, R.; Contreras-Manzano,

589 A., Nutritional status of iron, vitamin B12, folate, retinol and anemia in children 1 to

590 11 years old: Results of the Ensanut 2012. *Salud publica de Mexico* **2015,** *57* (5),

591 372-84.

592 48. Reeves, P. G.; Nielsen, F. H.; Fahey, G. C., Jr., AIN-93 purified diets for

593 laboratory rodents: final report of the American Institute of Nutrition ad hoc writing

594 committee on the reformulation of the AIN-76A rodent diet. *The Journal of nutrition*

595 **1993,** *123* (11), 1939-51.

Figure Captions

Figure 1. Growth performance of rats fed casein, soy, red meat and white meat protein diets.

A. IBW: initial body weight. B. FBW: final body weight. C. DBWG: daily body weight gain. D. DFI: daily feed intake. E. DFI/DBWG: ratio of daily feed intake to daily body weight gain.

Values are shown as means ± SD. The numbers of biological repetitions of casein, soy and red meat and white protein groups were 10, 10, 20 and 20, respectively. Different letters above bars indicate significant difference at $P < 0.05$ analyzed by one-way ANOVA and LSD multiple comparisons.

Figure 2. Adipose tissue weight, liver weight, liver TC and TAG content of rats fed casein, soy, red meat and white meat protein diets.

A. EATW: absolute weight of epididymal adipose tissue; EATW/BW: relative weight of epididymal adipose tissue to body weight. B. LW: absolute weight of liver; LW/BW: relative weight of liver to body weight. C. TAG-L: triacylglycerol in the liver; TC-L: total cholesterol in the liver.

Values are shown as means ± SD. The numbers of biological repetitions of casein, soy and red meat and white protein groups were 10, 10, 20 and 20, respectively. Different letters above bars indicate significant difference at $P < 0.05$ tested by one-way ANOVA and LSD multiple comparisons.

Figure 3. Plasma triacylglycerol, cholesterol, glucose and insulin concentrations of rats fed casein, soy, red meat or white meat protein diets.

A1. TAG: triacylglycerol. A2. TC: total cholesterol. A3. HDL-C: high density

lipoprotein-cholesterol. A4. LDL-C: low density lipoprotein-cholesterol. B1. Glucose. B2. Insulin. B3. HOMA-IR = [glucose (mmol/L) × insulin (mIU/L)/22.5], using fasting values.

Values are shown as means ± SD. The numbers of biological repetitions of casein, soy and red meat and white protein groups were 10, 10, 20 and 20, respectively. Different letters above bars indicate significant difference at $P < 0.05$ tested by one-way ANOVA and LSD multiple comparisons.

Figure 4. Plasma transaminase, total protein and urea concentrations of rats fed casein, soy, red meat or white meat protein diets.

A1. ALT: alanine aminotransferase. A2. AST: aspartate aminotransferase. A3. AST/ALT: ratio of aspartate aminotransferase to alanine aminotransferase. B1. TP: Total protein. B2: Urea.

Values are shown as means ± SD. The numbers of biological repetitions of casein, soy and red meat and white protein groups were 10, 10, 20 and 20, respectively. Different letters above bars indicate significant difference at $P < 0.05$ tested by one-way ANOVA and LSD multiple comparisons.

Table 1. Ingredient composition and nutritional content of diets

g/Kg diet	Casein	Soy	Pork	Beef	Chicken	Fish
diet composition, g/Kg diet						
Protein[1]	200	203	190	195	192	191
Cornstarch	398	398	398	398	398	398
Dyetros	132	132	132	132	132	132
Sucrose	100	100	100	100	100	100
Soybean oil	70	70	70	70	70	70
Cellulose	50	50	50	50	50	50
Mineral mix[2]	35.0	31.9	30.3	33.4	31.4	29.2
Vitamin mix[3]	10	10	10	10	10	10
L-Cystine[4]	3.0	0	0	0	0	0
Choline Bitartrate	2.5	2.5	2.5	2.5	2.5	2.5
nutritional level, U/Kg						
Energy，Kcal	4056	4056	4056	4056	4056	4056
Protein, g	177	177	177	177	177	177
Fat，g	70	70	70	70	70	70
Carbohydrate, g	680	680	680	680	680	680

Protein[1], the amount of protein powder was adjusted and balanced according to the protein content in soy and meat protein powder. Mineral mix [2], the formulation of mineral mixes for the six diets was listed in the Supplemental Table 1 online. Vitamin mix[3]: the formulation of vitamin mix was referenced to the paper[48]. L-Cystine[4]: the amino acid composition of soy and meat protein diets were not modified.

Table 2. Plasma amino acid concentrations of rats fed casein, soy, red meat or white meat protein diets.

μmol/L	casein n=10	soy n=10	white meat n=20	red meat n=20
TAA	3609±349^{b}	2586±220^{c}	4058±416^{a}	3527±617^{b}
EAA	2030±255^{b}	1265±129^{c}	2412±332^{a}	2102±381^{b}
NEAA	1579±102^{a}	1321±110^{b}	1646±151^{a}	1424±261^{b}
Val	207±38.1^{a}	121±18.4^{c}	189±25.6^{a}	160±29.4^{b}
Ile	99±15.18^{a}	67±15.8^{c}	95.4±14.4^{a}	82.0±15.9^{b}
Leu	148±24.4^{a}	88.7±17.9^{c}	135±22.8^{a}	113±24.0^{b}
Lys	581±92.6^{a}	355±81.2^{c}	576±102^{a}	466±86.8^{b}
Met	82.0±8.16^{a}	48.9±9.85^{c}	76.9±11.9^{a}	65.8±9.77^{b}
Phe	56.9±5.6^{b}	36.0±8.81^{c}	66.7±7.52^{a}	52.1±14.3^{b}
Thr	653±133^{b}	370±34.1^{c}	1037±194^{a}	963±208^{a}
His	73.2±7.70^{a}	64.8±6.89^{ab}	73.5±9.29^{a}	62.1±11.20^{b}
Arg	131±14.9^{b}	115±14.4^{b}	162±28.5^{a}	138±30.3^{b}
Pro	318±42.3^{b}	328±29.5^{b}	374±39.5^{a}	334±55.6^{b}
Tyr	99.1±11.0^{a}	60.7±9.95^{b}	101±20.0^{a}	90.9±21.9^{a}
Asp	21.5±4.93^{a}	12.8±4.17^{b}	16.2±5.42^{b}	13.5±7.55^{b}
Glu	127±25.3^{a}	75.1±15.8^{b}	87.4±13.6^{b}	85.4±24.5^{b}
Ala	466±59.7^{a}	264±42.0^{c}	400±97.6^{b}	326±71.9^{c}
Ser	256±30.7^{b}	269±21.5^{b}	320±35.1^{a}	267±51.3^{b}
Gly	280±44.3^{b}	294±30.1^{ab}	335±38.9^{a}	291±51.2^{b}
Cys	14.3±3.2	18.1±6.4	16.2±2.57	16.7±6.5

Values are shown as means ± SD. The different superscript letters within the same column mean statistical significant difference at $P < 0.05$ analyzed by one-way ANOVA and LSD multiple test. TAA: the sum of 17 kinds of amino acids in plasma including Arg, Pro, Met, Val, Ser, Gly, Lys, Thr, Phe, Asp, Ile, Leu, Cys, Glu, Ala, Tyr, His. EAA: the sum of 9 kinds of essential amino acids in plasma including Arg, Met, Val, Lys, Thr, Phe, Ile, Leu, His. NEAA: the sum of 8 kinds of non-essential amino acids in plasma including Pro, Ser, Gly, Asp, Cys, Glu, Ala, Tyr.

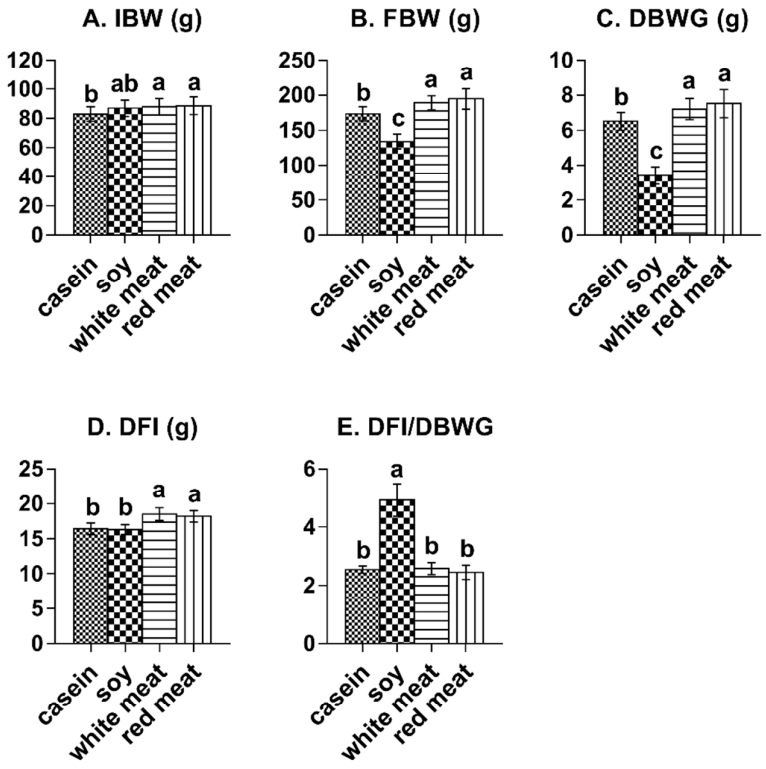
Table 3. Liver protein expression changes of rats fed casein, soy, red meat or white meat protein diets.

ID	symbol	protein name	casein	soy	white meat	red meat	GO BP	GO MF	GO CC
P15999	ATP5A1	ATP synthase subunit alpha	1.00^{b}	1.54^{a}	1.55^{a}	1.45^{a}	ATP synthesis	ATPase activity	mitochondrion
P13221	GOT1	Aspartate aminotransferase	1.00^{b}	2.22^{a}	1.73^{ab}	1.06^{b}	amino-acid biosynthesis	aminotransferase	cytoplasm
P00481	OTC	Ornithine carbamoyltransferase	1.00^{b}	1.83^{a}	0.78^{b}	0.71^{b}	urea cycle	transferase	mitochondrion
Q02253	ALDH6A1	Methylmalonate-semialdehyde dehydrogenase [acylating]	1.00^{ab}	1.32^{a}	0.87^{b}	1.06^{ab}	valine metabolic process	oxidoreductase	mitochondrion
P13444	MAT1A	S-adenosylmethionine synthase isoform type-1	1.00^{ab}	1.26^{a}	0.82^{b}	0.80^{b}	methionine metabolic process	transferase	cytoplasm
P62630	EF1A1	Elongation factor 1-alpha 1	1.00^{b}	1.45^{a}	1.07^{b}	0.94^{b}	protein biosynthesis	elongation factor	cytoplasm
P19112	FBP1	Fructose-1,6-bisphosphatase 1	1.00^{b}	1.61^{a}	0.87^{b}	0.72^{b}	gluconeogenesis	hydrolase	cytoplasm
P12346	TF	Serotransferrin	1.00^{b}	1.85^{a}	1.86^{a}	1.27^{b}	iron ion transport	ferrous iron binding	extracellular space
Q63716	PRDX1	Peroxiredoxin-1	1.00^{c}	1.83^{a}	1.41^{b}	1.24^{bc}	response to oxidative stress	peroxiredoxin activity	cytoplasm
Q9WVK7	HADH	Hydroxyacyl-coenzyme A dehydrogenase	1.00^{bc}	1.57^{ab}	1.65^{a}	0.86^{c}	fatty acid beta-oxidation	oxidoreductase	mitochondrion
P18163	ACSL1	Long-chain-fatty-acid--CoA ligase 1	1.00^{b}	1.10^{ab}	1.46^{a}	1.29^{ab}	fatty acid metabolic process	ligase	mitochondrion
D4A1W8	MTTP	Microsomal triglyceride transfer protein	1.00^{b}	1.07^{ab}	1.46^{a}	1.40^{ab}	lipoprotein transport	lipid transporter activity	plasma membrane
P24329	TST	Thiosulfate sulfurtransferase	1.00^{b}	0.97^{b}	1.36^{a}	0.99^{b}	sulfur amino acid catabolic process	transferase	mitochondrion
P06757	ADH1	Alcohol dehydrogenase 1	1.00^{b}	1.05^{b}	1.59^{a}	1.35^{ab}	acetaldehyde biosynthetic process	oxidoreductase	cytoplasm

Q6UPE0	CHDH	Choline dehydrogenase	1.00^{b}	1.26^{ab}	1.50^{a}	1.27^{ab}	choline oxidation process	oxidoreductase	mitochondrion
Q5XIH3	NDUFV1	NADH dehydrogenase (Ubiquinone) flavoprotein 1	1.00^{b}	1.59^{ab}	1.65^{a}	1.35^{ab}	electron transport	NAD binding	mitochondrion
P04636	MDH2	Malate dehydrogenase	1.00^{b}	1.44^{ab}	1.67^{a}	1.93^{a}	tricarboxylic acid cycle	oxidoreductase	mitochondrion
P04762	CAT	Catalase	1.00^{b}	1.38^{ab}	1.83^{a}	1.91^{a}	hydrogen peroxide catabolic process	catalase activity	peroxisome
P04642	LDHA	L-lactate dehydrogenase A chain	1.00^{bc}	0.86^{c}	1.23^{ab}	1.39^{a}	lactate metabolic process	oxidoreductase	cytoplasm
P12928	PKLR	Pyruvate kinase PKLR	1.00^{b}	0.66^{c}	1.14^{b}	1.38^{a}	glycolysis	kinase	cytoplasm
Q68FS4	LAP3	Cytosol aminopeptidase	1.00^{a}	0.55^{b}	0.90^{a}	0.95^{a}	proteolysis	aminopeptidase	cytoplasm
D3ZZL9	GCC2	GRIP and coiled-coil domain-containing protein 2	1.00^{a}	0.65^{b}	0.95^{a}	0.96^{a}	protein transport	protein binding	cytoplasm
O35077	GPD1	Glycerol-3-phosphate dehydrogenase [NAD(+)]	1.00^{ab}	0.64^{c}	0.94^{b}	1.13^{a}	triglyceride biosynthesis	oxidoreductase	cytoplasm
P16638	ACLY	ATP-citrate synthase	1.00^{ab}	0.54^{b}	1.18^{a}	1.38^{a}	lipid biosynthetic process	transferase	cytoplasm
P97532	MPST	3-mercaptopyruvate sulfurtransferase	1.00^{a}	0.93^{a}	0.64^{b}	0.60^{b}	transsulfuration	transferase	cytoplasm

Protein expression changes were represented as fold changes. The different superscript letters within the same column mean statistical significant difference at $P<0.05$ analyzed by one-way ANOVA and LSD multiple comparison of protein spots intensities (Supplementary Table 1). The numbers of biological repetitions of 2-DE analysis of casein, soy and red meat and white protein groups were 5, 5, 10 and 10, respectively. GO-BP: Gene Ontology-biological process; GO-MF: Gene Ontology-molecular function; GO-CC: Gene Ontology-cellular component.

Figure 1


A. IBW (g)
120
100
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white meat
red meat
B. FBW (g)
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white meat
red meat
C. DBWG (g)
10
8
6
4
2
0
b
c
a
a
casein
soy
white meat
red meat
D. DFI (g)
25
20
15
10
5
0
b
b
a
a
casein
soy
white meat
red meat
E. DFI/DBWG
6
4
2
0
b
a
b
b
casein
soy
white meat
red meat

Figure 2

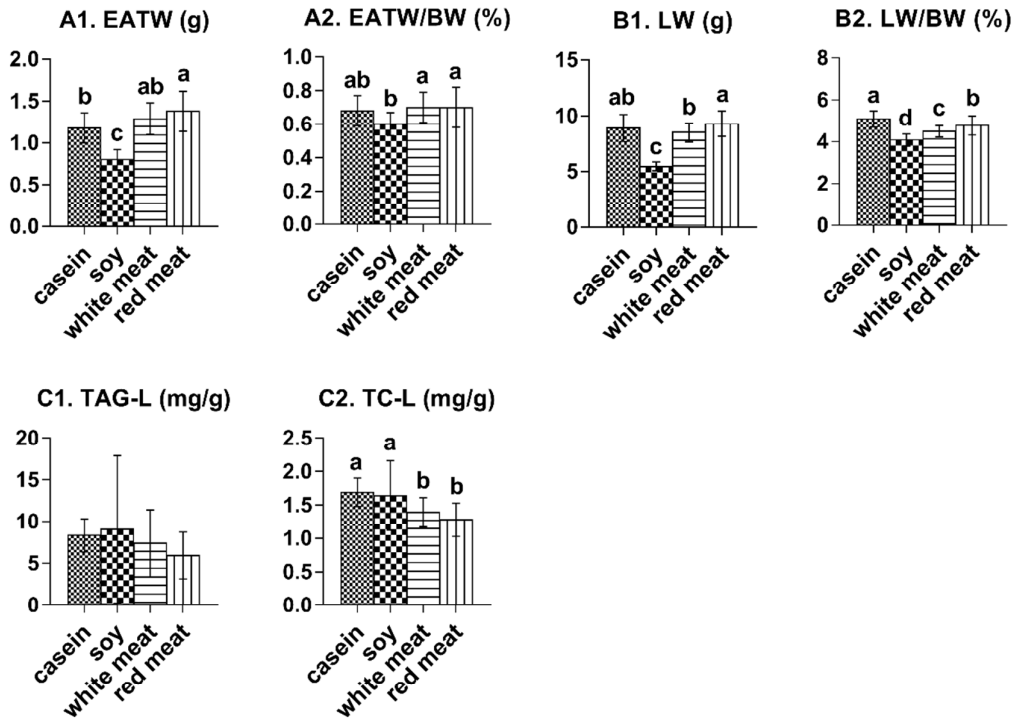

A1. EATW (g)
A2. EATW/BW (%)
B1. LW (g)
B2. LW/BW (%)
C1. TAG-L (mg/g)
C2. TC-L (mg/g)
casein
soy
white meat
red meat

Figure 3

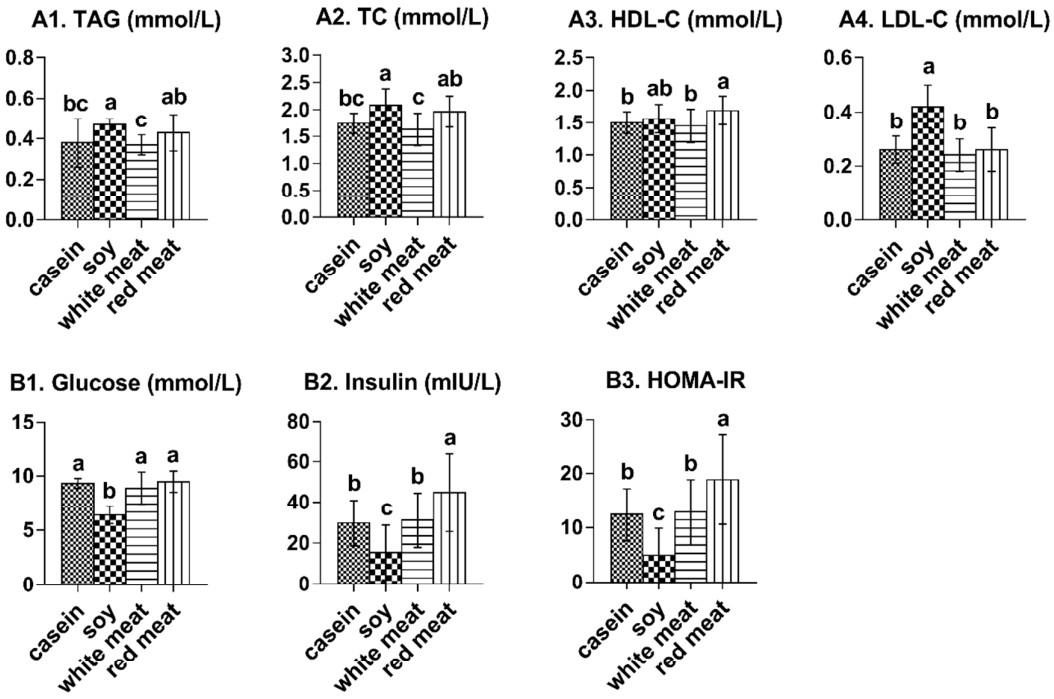
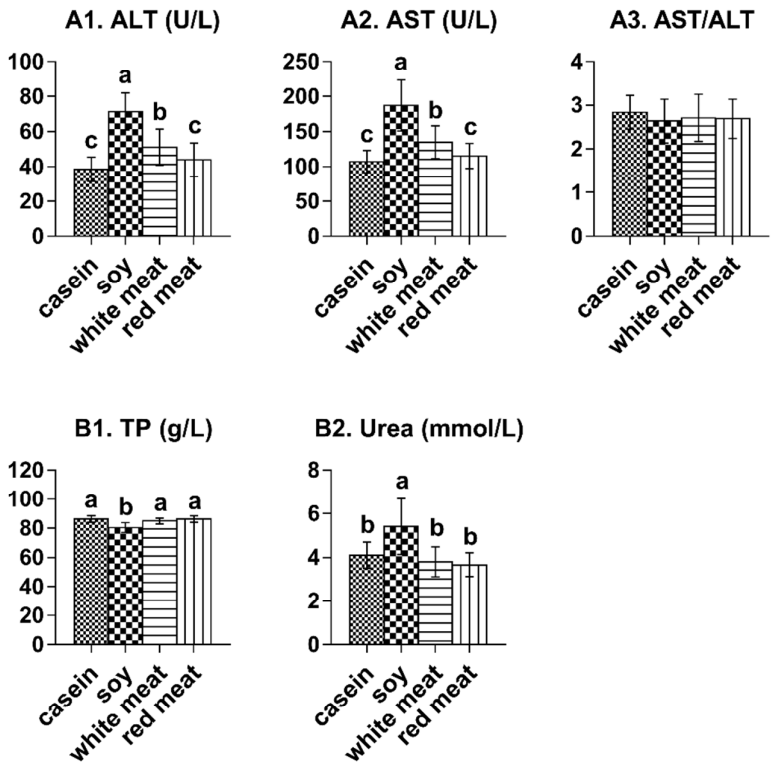

A1. TAG (mmol/L)
A2. TC (mmol/L)
A3. HDL-C (mmol/L)
A4. LDL-C (mmol/L)
B1. Glucose (mmol/L)
B2. Insulin (mIU/L)
B3. HOMA-IR
casein
soy
white meat
red meat

Figure 4


A1. ALT (U/L)
A2. AST (U/L)
A3. AST/ALT
B1. TP (g/L)
B2. Urea (mmol/L)
casein
soy
white meat
red meat

Graphic for table of contents