Brazilian Journal of Analytical Chemistry
2024, Volume 11, Issue 45, pp 56-109
doi: 10.30744/brjac.2179-3425.AR-34-2024

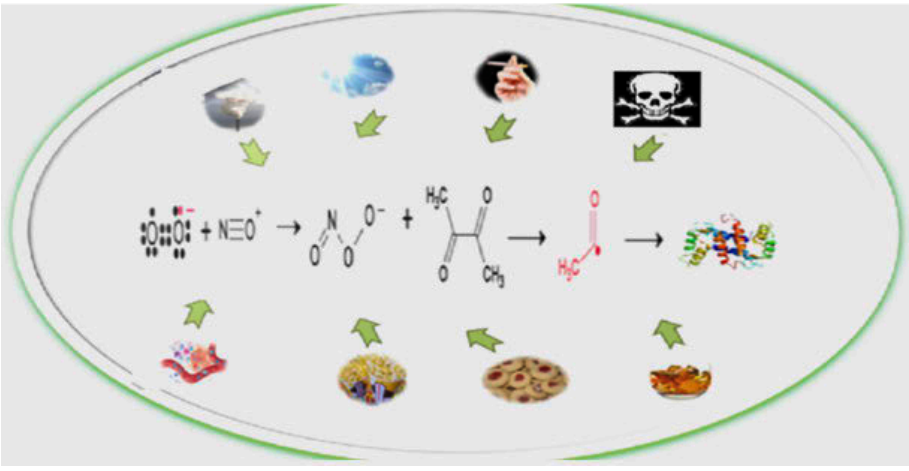
BrJAC

ARTICLE

Evidence of Increase in the Oxidative Stress in C57BL Mice Subjected to Daily Diacetyl Treatment: Oxidative Stress in Mice Subjected to Diacetyl Treatment

Letícia Dias Lima Jedlicka[1], Danilo Santos Souza[2], Danielle Zildeana Sousa Furtado[3,4], Lucas Ximenes Araújo[5,6], Emanuel Carrilho[5,6], Nilson Antonio Assunção[7]*

[1]*Instituto de Estudos em Saúde e Biológicas, Universidade Federal do Sul e Sudeste do Pará, UNIFESSPA, Campus III, Marabá, PA, Brazil*
[2]*Departamento de Agroindustria do Sertão, Universidade Federal de Sergipe, Rodovia Engenheiro Jorge Neto, km 3, Silos, 49680-000, Nossa Senhora da Glória, SE, Brazil*
[3]*Escola Paulista de Medicina, Universidade Federal de São Paulo, UNIFESP, Rua Botucatu, 740, Vila Clementino, 04023-062, São Paulo, SP, Brazil*
[4]*Faculdade de Tecnologia de Teresina, Rua Rio Grande do Norte, 790, Pirajá, Teresina, 64003-420, PI, Brazil*
[5]*Instituto de Química de São Carlos, Universidade de São Paulo, Avenida Trabalhador Sancarlense, Parque Arnold Schimidt, 13566-590, São Carlos, SP, Brazil*
[6]*Instituto Nacional de Ciência e Tecnologia em Bioanálise, INCTBio, Campinas, SP, Brazil*
[7]*Departamento de Química, Instituto de Ciências Ambientais, Químicas e Farmacêuticas, Universidade Federal de São Paulo, Rua Prof. Artur Riedel, 275, Jd. Eldorado, 09972-270, Diadema, SP, Brazil*



The food industry commonly utilizes diacetyl as a flavoring agent. However, concerns have recently emerged regarding its potential to cause adverse health effects, especially in individuals with frequent exposure. In this study, our objective was to assess the impacts of diacetyl on male mice by exposing them to diacetyl through drinking water for 15 days, thereby simulating the consumption of diacetyl-containing products. Our study focused on crucial parameters associated with dicarbonyl stress, specifically evaluating methylglyoxal, glutathione synthetase, and glutathione levels in liver tissue using LC-MS. We also analyzed changes in concentrations of plasmatic proteins. Our findings indicated a decrease in

Cite: Jedlicka, L. D. L.; Souza, D. S.; Furtado, D. Z. S.; Araújo, L. X.; Carrilho, E.; Assunção, N. A. Evidence of Increase in the Oxidative Stress in C57BL Mice Subjected to Daily Diacetyl Treatment: Oxidative Stress in Mice Subjected to Diacetyl Treatment. *Braz. J. Anal. Chem.* 2024, *11* (45), pp 56-109. http://dx.doi.org/10.30744/brjac.2179-3425.AR-34-2024.

Submitted March 12, 2024; Resubmitted August 01, 2024; Accepted August 06, 2024; Available online August 29, 2024.

methylglyoxal levels despite increased carbonyl compounds and the glutathione / glutathione synthetase ratio, implying significant alterations in the proteomic profile due to diacetyl consumption. Subsequent analysis identified nineteen differentially expressed proteins, with fifteen suppressed proteins in both groups exposed to diacetyl. Gene ontology analysis revealed that these proteins play roles in various biological responses and metabolic processes, underscoring the potential health risks associated with daily diacetyl consumption. This study emphasizes the necessity for further research and consideration of diacetyl's potential adverse health effects, mainly when used as a flavoring agent in food products.

Keywords: methylglyoxal, glutathione, proteomic, carbonyl stress, diacetyl

INTRODUCTION

Diacetyl is an α-dicarbonyl compound present in a variety of foods such as latkes (potato pancakes), wine, beer, cake, microwave popcorn, and biscuits.[1–6] It may be naturally present in these foods[7] or be added for the purpose of enhancing or improving the taste and/or aroma.[6,8,9] This compound is also found in conventional cigarettes and has recently been employed in the formulation of electronic cigarettes.[10,11]

Previous *in vitro* studies conducted by our group have demonstrated that diacetyl reacts with peroxynitrite, which is an endogenous substance whose production is increased in stress situations.[11–15] In the present study, the formation of a high concentration of acetyl radicals was demonstrated *in vitro* through a nucleophilic addition reaction of peroxynitrite with 2,3-butanedione in phosphate buffer (pH 7.2).

Previously we shown, by the addition of L-histidine or 2'-deoxyguanosine to the reaction medium, that acetyl radicals generated from the reaction between 2,3-butanedione and peroxynitrite promote the acetylation of proteins and nitrogenous bases.[12,14] Another *in vitro* study by our group also proved the production of acetyl radicals and the formation of acetylated products via magnetic resonance and mass spectrometry data, which was due to covalent bonds between the acetyl radicals and amino acids, peptides, and proteins.[11–15] *In vivo* studies have also been performed in Wistar rats and C57BL mice demonstrating that the daily intake of diacetyl causes significant changes in the protein, including increased protein acetylation.[16,17] The increase in acetyl radicals contributes to the redox imbalance of the organism; one of the defense mechanisms is the increased production and consumption of antioxidant substances by the body,[18] such as glutathione.[19,20] Another study, *in vivo*, of our group showed that diacetyl intake is capable to modify plasmatic metabolic profile in mices C57/Bl, this study also demonstrated that there were responses between males and females who ingested daily diacetyl, can lead to a predisposition to different diseases depending on the sexes and also leave to metabolic alterations.[17]

Methylglyoxal (MGO) is α-dicarbonyl compound present in cells and is an important precursor of free radicals, as well as being an intermediate in cellular metabolism under normal and pathological conditions.[21,22] Three metabolic routes of MGO formation in mammals are known; one suggests the formation of MGO from phosphate trioses, and another, from ketone bodies. These two routes account for 90% of the MGO formed in the body. A third route leads to the formation of MGO from glycine and threonine, with the previous formation of amino acids.[23] The increased in MGO production can lead to the formation of carbonyl stress revealed that carbonyl stress by chronic exposure to MGO is associated altered GSH/GSSG redox potential.[24] In addition, the reactions involved in the formation of carbonyl species are related with increase the formation of reactive oxygen species (ROS). It can lead to increase in oxidative stress and consequent structural and functional damage to macromolecules such as proteins.[25] And the proteins play an important role in most processes biological, they are repatriated by various biological and molecular functions like, for example: catalysis, transport, connection to substances, function signaling, hormonal, among others. Mass spectrometry is used in most studies in proteomics, being responsible for most of protein identification.[26]

MATERIALS AND METHODS

Reagents and standards

Trichloroacetic acid P.A (TCA) (Merck, Germany), trifluoroacetic acid (TFA) chromatographic grade, acetonitrile MS grade (Sigma-Aldrich, Germany), and formic acid HPLC grade (Sigma-Aldrich, Germany) were used. For quantitation: glutathione (GSH), methylglyoxal, tryptophan, o-phenylene diamine (DB) and oxidized glutathione (GSSG) (Sigma-Aldrich, Germany). Diacetyl, heparin, and acid trifluoroacetic were purchased from Sigma Aldrich (MO, USA). Bradford reagent, Dithiothreitol (DTT) and iodoacetamide (IAA) were purchased from Biorad, USA and trypsin from Promega, USA.

Mouse model development

All procedures used in the present study were performed in accordance with the guidelines established by the Brazilian College of Animal Experimentation (COBEA) and were approved by the Ethical Committee of the School of Medicine of the Federal University of São Paulo (UNIFESP); protocol No. 975911013/16.

Ten- to twelve-week-old male C57BL mice were distributed into three groups, each consisting of six animals. These mice were chosen exclusively as males to avoid the hormonal variability present during the females' estrous cycle, which can affect the accuracy of the data collected. [27] The animals were housed in cages under controlled conditions with 50−70% humidity, a temperature range of 19-26 °C, and a 12-hour light/12-hour dark cycle and were fed *ad libitum.* The mice received two different doses of diacetyl, based on the reference study by Colley et al., for 15 weeks.[28] The treatment groups were as follows: a control group that received only drinking water; a group that received 200 mg (diacetyl) per kg (animal weight) per day in the drinking water; and a group that received 300 mg kg^{-1} day^{-1} in the drinking water. Following 15 weeks of treatment, the animals were anesthetised with CO_2 and sacrificed. The livers were removed and stored at -80 °C until use. The blood was collected in a tube with heparin that was centrifuged, and the plasma was collected and stored -80 °C freezer.

GSH and GSSG analysis

GSH and GSSG extraction was performed based on the methodology described by Mika *et. al* [22] with some adaptations. Firstly, an aliquot of approximately 50 mg hepatic tissue was placed in a microfuge tube, and a 1:4 (v/v) solution of trichloroacetic acid (10%) was added, followed by immediately homogenization using a vortex for 1 min. The solution was subsequently centrifuged at 10,000 x g for 10 min at 4 °C, and the supernatant was collected and diluted (1:200) with the mobile phase (0.1% formic acid−H_2O) prior to HPLC.

For both glutathione and methylglyoxal analysis, liquid chromatography (LC) was operated using a UFLC XR (Shimadzu, Japan) automatic injector equipment, coupled to a mass spectrometer with a linear ion trap analyzer and electrospray (ESI) ionization source (Amazon model; Bruker, Germany). Separation of the analytes occurred on a C18 reversed-phase analytical column (100 mm x 3 mm, 2.5 µm) (Shim-Pack XR ODS-II). A C18, 4.6 x 12.5 mm guard column (Eclipse XDB-C18 4-Pack, Zorbax-Agilent Technologies, USA) was coupled to the methylglyoxal determination system.

The mobile phase was composed by water and 0.1% formic acid (Phase A) and ACN and 0.1% formic acid (Phase B). A volume of 5 µL sample was injected, and the gradient started with 1% solvent B, increasing to 3% over 2.5 min, 30% over 3 min, and 35% over 40 min. A decrease to 30% and 1% occurred over 7 and 9 min, respectively, and was maintained for an additional 6 minutes for stabilization. The flow was maintained constant at 0.2 mL min^{-1} and the column temperature at 40 °C. The ions [M + 1] of GSH and GSSG generated from the ESI were analyzed in IT ultra scan mode, the masses were 308.1 *m/z* and 613 *m/z*, respectively. The parameters established were ESI voltage, 4500 V; nebulizer pressure, 1.2 bar; gas flow, 6.0 L min^{-1}; temperature of the source, 180 °C; and scanning range, 110−700 *m/z*. The Supplementary material (Table S1-S2) present the results for the parameters evaluated during the validation of the method for the separation and quantitation of GSH and GSSG.

Methylglyoxal analysis

Preparation of the samples for methylglyoxal analysis was performed using tissue based on the protocol described by Rabbani and Xue.[29] Approximately 50 mg hepatic tissue was homogenized in 10 µL TCA saline (4% + 0.9% NaCl) using a vortex at 6 °C for 10 seconds, followed by the addition of 80 µL H_2O (Milli-Q). Subsequently, 5 µL tryptophan standard (13C13 and 15N) (400 nM) was added. Following further vortex, the mixture was centrifuged (1000 x g, 4 ºC) for 10 min, and 35 µL supernatant was transferred to the inserts.

The methylglyoxal derivatization process was carried out by the addition of 5 µL sodium azide (3% w/v) and 10 µL DB derivatization reagent (0.5 nM) to the insert, to a final volume of 50 µL. The mixture was incubated for four hours in the dark at room temperature. After the incubation period, the extract was subjected to liquid chromatography mass spectrometry (LC-MS) analysis with a total injection of 50 µL to determine the derivatization product (2-methylquinoxaline − 2MQ).

The conditions for the separation of methylglyoxal were as described by Rabbani and Thornalley, 2014,[23] using mobile phase A, containing 0.1% (v/v) TFA in water, and mobile phase B containing 0.1% (v/v) TFA with 50% (v/v) water and HPLC grade acetonitrile. The samples were maintained at 4 ºC in the autosampler. A volume of 50 µL sample was injected, and a flow of 0.2 mL min^{-1} 0% mobile phase A was started and maintained for 0.5 min. Subsequently, a linear gradient of 0 to 100% phase B was started over 10 min, which was maintained for 5 min and reduced to the initial 0% phase B at 16 min. These conditions were maintained for a further 15 min to achieve rebalancing and column washing. Thus, a 31-min chromatographic run was performed. The internal tryptophan and 2MQ (2-methylquinoxaline-2MQ) standards were ionized by the ESI in positive mode [M + 1] and monitored in ultra scan mode on the ion trap in the range of 100−300 *m/z*. The ions 207.2 *m/z* and 145.2 *m/z* for labeled tryptophan (1C13 and N15) and 2MQ, respectively, were extracted. The other conditions of the mass spectrometer were set at: ESI voltage, 4500 V; temperature of the source, 180 °C; end plate offset voltage, 500 V, gas pressure, 1.2 bar; and gas flow, 6.0 L min^{-1}. The summary of the of the analysis of variance (ANOVA) of the linear model used in the quantification of methylglyoxal is described in supplementary material (Table S3).

The quantification of methylglyoxal was carried out using a significantly linear calibration curve (p<0.05) with no lack of fit. The concentration range of the 2-MQ standard used was from 2.0 to 20.0 pmol L^{-1}, with points executed in triplicate, and the peak areas were obtained after chromatogram integration. For quantification in liver samples, tryptophan was used as an internal standard (IS) with a fixed concentration of 8 pmol L^{-1} to be subtracted from the total amount measured in the samples.

Proteic Carbonyl Analysis

Carbonyl determination compounds was carried out according to the method described by Levine and collaborators with some minor adjustments as follow above.[30] The carbonyl groups analyzed in the plasma samples were first derivatized with 2,4-dinitrophenylhydrazone (DNP) by reaction with 2,4-dinitrophenylhydrazine (DNPH), in the presence of 2.5 M HCl. The samples were incubated in the dark, at room temperature, for 1 hour, shaking the tubes every 15 minutes. Then, a 20% solution of TCA was added to the test and white tubes. Both tubes were incubated on ice for 10 minutes and centrifuged at 12000 rpm for 10 min at 4 ºC, the supernatant was discarded, and the pellet was washed with 10% TCA solution. The pellet was washed four more times, with a 1:1 solution of ethanol and ethyl acetate. The pellets were resuspended in 6.0 M guanidine solution and incubated at 37 °C for 10 minutes. The samples were quickly centrifuged with a spin, analyzed by spectrophotometry and the results were expressed in nanomoles of carbonyl per milligram of protein.

Protein Analysis

The total protein concentration in plasma was determined by the Bradford method.[31] Than, 50 µg of protein from plasma were digested separately, first were added Rapid Digest (PN: 186001861. Waters, USA) reduced with DTT (Biorad, USA) and alkylated with iodoacetamide (Biorad, USA). Trypsin

(Promega, USA) was added at a ratio of 1:100 enzyme: protein, and the reaction was stopped by adding TFA (Sigma Aldrich). The sample was dried by Speed Vacuum concentrators to reduce the volume to approximately 50 µL, was added 30 µL 0.5% TFA. The digested samples were filtered with a PVDF 0.22 µm syringe filter (PN SLGV033RS; Millipore Millex-GV, Ireland) the sample was stored at 4 °C until mass spectrometry analysis.

Three replicates of digested plasma samples were analyzed by mass spectrometry in SYNAPT G2 HDMS (Waters, USA) coupled Nano-Acquity HPLC (Waters, USA) equipped with a binary pump, autosampler, and a thermostatically controlled column compartment. In Nano-LC utilize a C18 reversed phase column Acquity UPLC Nano-CSH with dimensions 130 x 200 mm, 75 µm particle size of 1.7 µM (PN: 186 007 072; Waters, USA) and a trap nano-column Acquity BEH C18 130 µM with dimensions 180 x 20 mm, 5 µm particle size (BP: 186 003 682; Waters, US). The column temperature was maintained at 40 °C. Samples were separated using a mobile phase gradient composed of (A) formic acid/H_2O (1:1000) and (B) Formic acid/ACN (1:1000) and an elution gradient 7-35% B over 90 min. The flow rate was set 275 nL min^{-1} and the injection volume was 5 µL. The acquisitions were performed using the SYNAPT G2 mass spectrometer (Waters, USA) equipped with an ESI. The mass spectrometer was operated in positive mode at 3.0 kV, desolvation temperature 70 °C, the drying gas (N_2) at a flow rate of 4 L min^{-1}. All acquisition transactions were controlled by MassLynx software version 4.1 (Waters, USA) for analysis MS/MS method was used data-independent acquisition (DIA) and CID fragmentation with argon gas. Mass spectra were collected between 50 and 1600 *m/z* and total time of 1.25 s scan. The calibration was performed with [Glu-1]-fibrinopeptide b-500 fmol $µl^{-1}$ (PN: 700 004 729; Waters, USA) and flow of 500 nL min^{-1} acquired for 1 s every 60 s. The low collision energy was 4 eV and the high-energy collision ranged 17-60 eV.

Bioinformatic analysis

Data files were processed by ProteinLynx Global Service (PGLS) (version 3, Waters, Milford, MA, USA) with limits for low scores, high energy, and intensity of 50 and 750 and 1200, respectively, resolution of the time of flight (TOF) and width of automatic peaks *m/z* from calibrant. The searches against the database were performed with sequences of *mus musculus* from Uniprot.[32] The following parameters were adopted: cysteine carbamidomethylation as fixed modification and lysine acetylation, histidine, arginine, methionine oxidation as variable modifications, and one missed cleavage and automatic precursor and fragment error tolerance. The limits used for identification were minimum one fragment by peptide, five fragments of protein, two fragments of peptide and maximal false positive rate of 5%, as estimated by simultaneous search with a reverse database.

Label free quantification

We preceded the Label-free quantification based on DIA workflows from PGLS. We use the protein top 3 Matched Peptide Intensity Sum (TOP 3), this parameter is calculated automatic by PGLS. And corresponding to the mean of the three highest peptides areas measured for each protein. Then, we divided the sum of all observed TOP 3 peptide intensities by the number of all observable peptides; this ratio provides a measure to approximate absolute protein concentration.[33,34]

Orthologs Analysis

We subject Orthologs to Gene Ontology (GO) term analysis-based PANTHER classification online tools.[35] This analysis was performed for determining the functions of proteins differentially expressed in group treated with diacetyl from control group. For this search we used the Uniprot IDs with *mus muscullus* genome.[36]

Such database was chosen as the reference database for the output report on biological process, and molecular function information.[32] For classification of pathways with these proteins we use the panther database.[35] These analyses were performed to get the profile of this proteins differentially expressed in group treated with diacetyl from control group.

Statistical analysis

The statistical analysis was based on statistical methods for comparing data through hypothesis testing ANOVA was applied followed by Tukey's test ($p \leq 0.05$) *a post hoc* test used to determine the significant differences between group means in an analysis of variance setting using GraphPad Prism (version 5.00 for Windows, GraphPad Software, San Diego California USA) to analyze the proteins concentration screening.

Additionally, principal component analysis (PCA) was applied to identify trends or similarities between samples as well as any correlation between variables, using the web toll Clustvies.[32,37]

RESULTS AND DISCUSSION

Quantitation of GSH and hepatic reduced glutathione (GSSG) in mouse liver

The results for the determined concentrations of GSH and GSSH in the livers of mice subjected to diacetyl in the diet are shown in Table I. It can be observed that the GSH content in mouse liver was higher than that of GSSH under all treatment conditions with the addition of diacetyl radicals as compared with mice that received no diacetyl in the diet. In this case, the GSH content was similar to the control, although there was a numerical difference with respect to the GSSG content (1.68 ± 0.57 and 1.03 ± 0.43 μg_{GSH} mg_{liver}^{-1} for GSH and GSSG, respectively). These results can be justified by the fact that livers from different mice were analyzed, which have different metabolisms despite being standardized under the same genetic and environmental conditions. This likely increased the variability of the data, and consequently increased the standard deviations, minimizing the statistical difference between the data.

Table I. Concentrations of GSH and GSSG in the livers of mice subjected to a diet containing diacetyl

Treatment	GSH (μg_{GSH} mg_{liver}^{-1})	GSSG (μg_{GSSG} mg_{liver}^{-1})
Control	$1.68 \pm 0.57^{aB*}$	1.03 ± 0.43^{aA}
200 mg	2.48 ± 0.61^{aA}	0.73 ± 0.22^{bA}
300 mg	2.02 ± 0.41^{aAB}	0.71 ± 0.26^{bA}

*Means followed by the same lowercase letter in the row and the same upper case in the column do not represent a significant difference at $p \leq 0.05$, according to Tukey's test.

It can also be seen from Table I, that the GSH content was increased in the livers of mice subjected to a diet containing diacetyl radicals (200 and 300 mg), rising from 1.68 μg_{GSH} mg_{liver}^{-1} for the control to 2.48 and 2.02 μg_{GSH} mg_{liver}^{-1} for treatment with 200 and 300 mg, respectively. On the other hand, the GSSG content was reduced in the livers of mice submitted to a diet containing diacetyl. Livers of mice that received no diacetyl in the diet contained approximately 1.03 ± 0.43 μg_{GSH} mg_{liver}^{-1}, whereas the livers of mice that received the dietary radical had a content of 0.73 and 0.71 μg_{GSH} mg_{liver}^{-1} for 200 and 300 mg treatment, respectively. Similar to the GSH concentration in mouse liver, the different diacetyl radical concentrations studied (200 and 300 mg) in the diet were insufficient to cause significant differences between them. It was only possible to observe significant differences when the data were compared with those of the control mice.

The results for the ratio between GSH and GSSG are shown in Figure 1A. It is noteworthy that there was a significant difference between the data obtained from the mice treated with the dietary diacetyl radicals and those obtained from the control mice. The GSH/GSSG ratio for the control group remained at approximately 1.60, and that for the groups treated with 200 and 300 mg diacetyl was approximately 2.4 and 3.9, respectively. Although these groups differed from the control, there was no significant difference between the two groups, as can be observed in Figure 1A.

Figure 1B shows the MGO levels in liver tissue, it can be observed that incorporation of diacetyl (200 and 300 mg) into the diet significantly reduced the content of MGO present in mouse liver ($p \leq 0.05$) as compared with the livers of the control mice that received no diacetyl in the diet. The livers of the mice in the control group had a mean MGO content of approximately 13.65 ± 3.33 $pmol_{MGO}$ mg_{liver}^{-1}, whereas those in the groups that received 200 and 300 mg diacetyl in the diet presented concentrations of approximately 9.27 ± 2.4 and 9.4 ± 2.4 $pmol_{MGO}$ mg_{liver}^{-1}, respectively. Therefore, the results show that treatment with diacetyl at these concentrations was insufficient to differentiate among the groups in relation to the MGO content in the liver, maintaining a significant equality ($p \leq 0.05$).

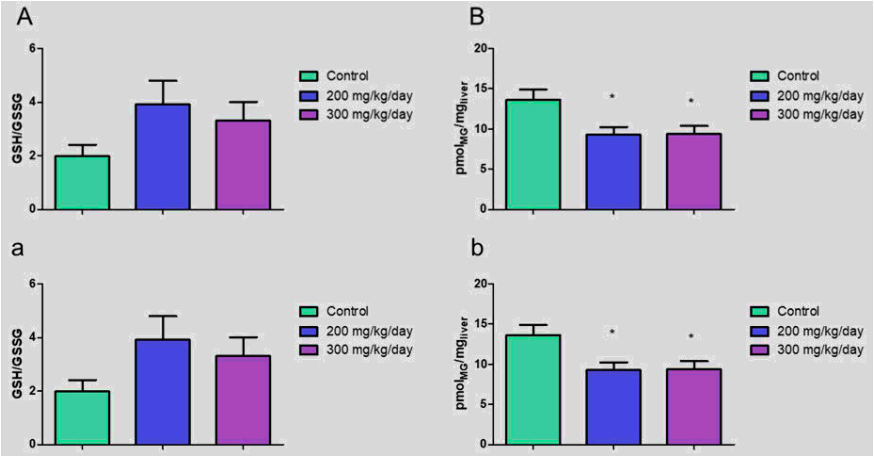


Figure 1. (A) Ratio of GSH/GSSG content determined in the livers of mice subject ted to daily intake of diacetyl. (B) MGO content determined in the livers of mice subjected to daily intake of diacetyl. *Significative difference from control group.

Carbonyl Stress analysis

The rise in the carbonyl species is a strong indicator to oxidative stress is increased, in this work we show this rise in carbonyl species in plasma from mouse treated with diacetyl. Also, we show when animals consumption more diacetyl the increased in carbonyl species is higher. (Figure 2)

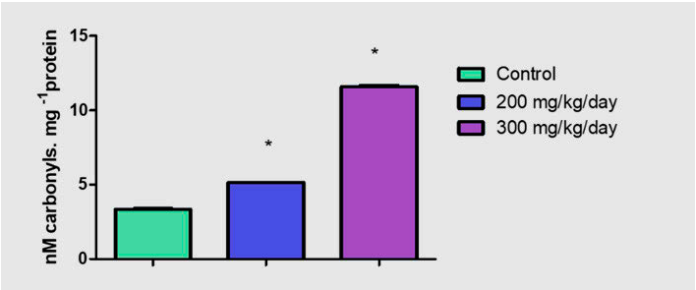


Figure 2. Carbonyl stress in plasma of mice subjected to daily intake of diacetyl. *Significative difference from control group.

PCA analyses and Heatmap from GSH, GSSG, MGO ant Carbonyl Stress

To ensure a cleaner and more coherent visualization of the data, PCA was conducted using average values due to the use of isogenic mice, which are genetically identical, and because the studies involved pooling samples from each group. This methodological choice aimed to minimize variability and present a more representative overview of the underlying patterns, reducing the potential for artifacts. The heatmap

(Figure 3a) displays the differences in substance levels, showing the separation between the control and diacetyl-treated groups. The rows are centered, and unit variance scaling is applied to the rows. Both rows and columns are clustered using correlation distance and average linkage, resulting in five rows and three columns. Despite the use of averages, the reliability and accuracy of the results were not compromised. The PCA analyses (Figure 3b) demonstrated that have significative difference and separation between the control group and the groups treated with 200 and 300 mg kg^{-1} day^{-1} of diacetyl. This analysis shows a perfect separation PC1 81% and PC2 19% totalizing 100%. Unit variance scaling is applied to rows; SVD with imputation is used to calculate principal components. X and Y axis show principal component 1 and principal component 2 that explain 81% and 19% of the total variance. The data preprocessing is available in the supplementary material (Tables S4-S6).

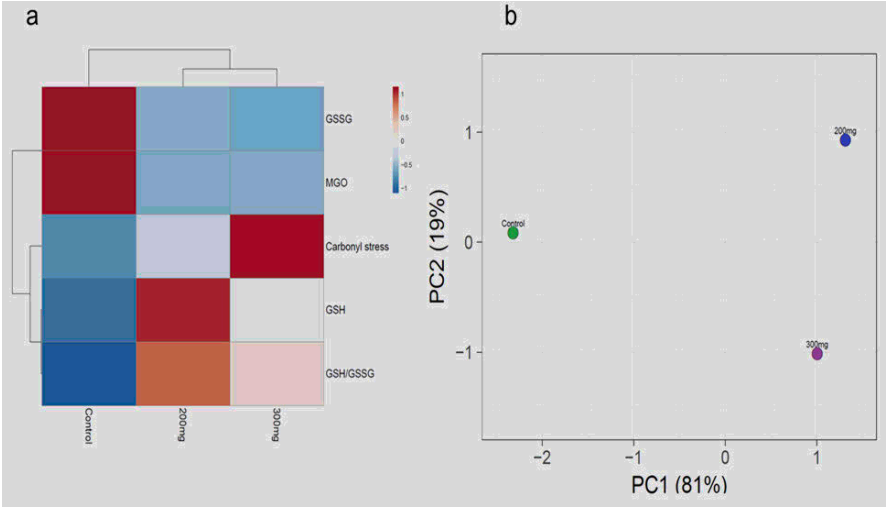


Figure 3. (a) Heatmap analyses from GSH, GSSG, MGO in liver tissue and Carbonyl Stress (carbonyl compounds in plasma); (b) PCA analyses from GSH, GSSG, MGO ant Carbonyl Stress; *(dot green corresponding to control group, dot blue corresponding to group treated with 200 mg kg $^{-1}$ day^{-1} of diacetyl and dot purple corresponding to group treated with 300 mg kg $^{-1}$ day^{-1} of diacetyl).

Proteomic Analysis

The proteomics analysis in plasma from groups control (in supplementary material Table S7) and treated with 200 (Table S8) and 300 mg kg $^{-1}$ day^{-1} (Table S9) show differences between the control and treated groups. As showed in Table II, two proteins (A2M and Albu) that are less expressed in treated groups. We also, identified four proteins (IDHC, KLKB1, KV6AB and PHLD) that are identified only in control group, and identified some proteins that are not identified in the group that received 300 mg kg $^{-1}$ day^{-1} of diacetyl (APOC3, B2MG, CBG, CBPN, CFAH, CPN2, FETUA, FETUB, HEP2, LUM and MBL2).

Table II. Relative quantification (%) of plasmatic proteins differentially expressed identified in control group and groups treated with 100, 200 and 300 mg kg $^{-1}$ day^{-1} of 2,3-butanedione

Protein Acess	Protein key	Control (fmol)	200 mg kg $^{-1}$ day^{-1} (fmol)	Compare to control group	300 mg kg $^{-1}$ day^{-1} (fmol)	Compare to control group
A2M	162	9.135 ± 1.525*	8.41 ± 2.0*	↔	3.67 ± 2.11*	↓
ALBU	846	21.878 ± 2.793*	20.96 ± 3.4	↔	12.061 ± 2.600*	↓
A1AT4	151	N/F	3.674 ± 0.26	↑	2.187 ± 1.543	↑

(continued on next page)

Table II. Relative quantification (%) of plasmatic proteins differentially expressed identified in control group and groups treated with 100, 200 and 300 mg kg $^{-1}$ day^{-1} of 2,3-butanedione (continued)

Protein Acess	Protein key	Control (fmol)	200 mg kg $^{-1}$ day^{-1} (fmol)	Compare to control group	300 mg kg $^{-1}$ day^{-1} (fmol)	Compare to control group
HA10	9187	N/F	0.152 ± 0.115	↑	0,404 ± 0.08	↑
IDHC	9991	0.113 ± 0.095	N/F	———	N/F	———
KLKB1	11379	0.118 ± 0.005	N/F	———	N/F	———
KV6AB	11609	0.046 ± 0.034	N/F	———	N/F	———
PHLD	16194	0,079 ± 0.020	N/F	———	N/F	———
APOC3	1273	0.043 ± 0.018	0.034 ± 0.027	↔	N/F	———
B2MG	1899	0.223 ± 0.103	0.221 ± 0.178	↔	N/F	———
CBG	2878	0.251 ± 0.054	0.241 ± 0.052	↔	N/F	———
CBPN	2931	0.059 ± 0.025	0.062 ± 0.014	↔	N/F	———
CFAH	3735	0.433 ± 0.110	0.327 ± 0.053	↔	N/F	———
CPN2	4710	0.114 ± 0.048	0.100 ± 0.042	↔	N/F	———
FETUA	7642	0.680 ± 0.132	0.991 ± 0.413	↔	N/F	———
FETUB	7643	0.320 ± 0.090	0.313 ± 0.086	↔	N/F	———
HEP2	9394	0.096 ± 0.020	0.095 ± 0.019	↔	N/F	———
LUM	12289	0.073 ± 0.008	0.085 ± 0.012	↔	N/F	———
MBL2	12678	0.140 ± 0.041	0.112 ± 0.052	↔	N/F	———

Mean ± SEM (N=3), ↑ increase comparing to control group or expressed only in treated group, ↓ decrease comparing to control group, ↔ no difference from control group, * significant difference between the proteins (lines) in the different groups (columns). ANOVA TWO ways *post hoc* test Bonferroni * P > < 0.0001.

The proteins differentially expressed in treaded groups belongs to different class (Figure 4a), including defense/immunity protein (PC00090), extracellular matrix protein (PC00102), protein-binding activity modulator (PC00095), transfer/carrier protein (PC00219), metabolite interconversion enzyme (PC00262), protein modifying enzyme (PC00260) and transmembrane signal receptor (PC00197). The Figure 4b shows that proteins are involved in three molecular functions indicated by panther dB as binding (GO:0005488), catalytic activity (GO:0003824) and molecular function regulator (GO:0098772). Already the biological process (Figure 4c) that proteins participates are biological regulation (GO:0065007), cellular component organization or biogenesis (GO:0071840), cellular process (GO:0009987), immune system process (GO:0002376), response to stimulus (GO:0050896), developmental process (GO:0032502), growth (GO:0040007), localization (GO:0051179), metabolic process (GO:0008152) and multicellular organismal process (GO:0032501). Furthermore, these proteins differentially expressed in treated groups are involved into three important pathways Blood coagulation (P00011), T cell activation (P00053) and Gonadotropin-releasing hormone receptor pathway (P06664) according to panther database analysis.[35]

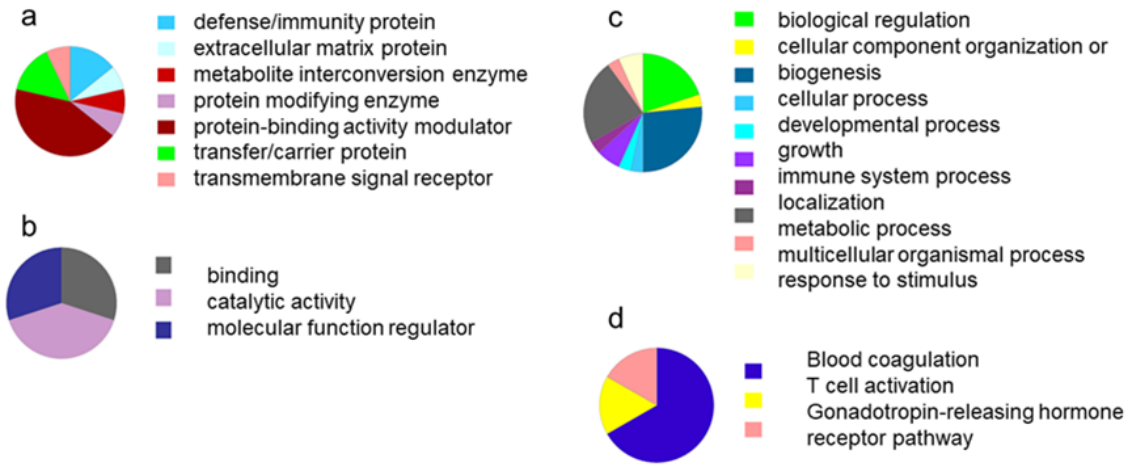


Figure 4. Go analysis from proteins with differential expression in groups treated with diacetyl: (a) Protein Class, (b) Metabolical process, (c) Biological process and (d) Pathways.

Discussion

The different diacetyl radical concentrations (200 and 300 mg) in the diet were insufficient to cause significant differences in GSH between the two groups; however, when the data were compared with those of the control mice, an increase in GSH was observed (Table I). Regarding the ratio between GSH and GSSG (Figure 1A), it is noteworthy that there was a significant difference between the data obtained in mice treated with the dietary diacetyl radicals and those obtained in mice in the control group. The GSH/GSSG ratio for the control group remained at approximately 1.60, and the ratio for the groups treated with 200 and 300 mg diacetyl was approximately 2.4 and 3.9, respectively. GSH is involved in many biological processes, such as detoxification of xenobiotics, and is important in animal tissues to help prevent injuries caused by reactive oxygen species such as free radicals and peroxides. Most of the glutathione in cells is in the form of GSH, but when cells suffer oxidative stress, the GSSG form accumulates, increasing the ratio of GSSG to GSH. An increased ratio of GSH/GSSG is an indication of oxidative stress.[38–40]

Similarities were noted between the 200 and 300 mg treatment samples, which is the result of lower concentrations of MGO (Figure 1B). In general, the control samples had great variation in the concentrations of MGO, which can be explained by the metabolic individuality of each animal studied. The decrease in MGO levels may be related to the increase in the GSH/GSSG ratio, suggesting that MGO may have been consumed or detoxified. Schmitz *et. al* have previously shown that there is a relationship between the methylglyoxal levels and the GSH/GSSG ratio.[41] Indeed, liver is the main responsible tissue for detoxification of MGO and glyoxal.[42,43] This liver detoxification ability may explain the decreased levels of MGO in the liver of animals from groups treated with diacetyl compared to the liver of animals from control group. Another condition that corroborates for MGO decrease is besides it being a very reactive compound the MGO has a largely renal excretion, and this mechanism can prevent the toxicity of MGO.[43] Intracellular MGO has been proven to be detoxified by the glyoxalase system. However, the details of this mechanisms for detoxification of MGO have not yet been fully elucidated.[43,44] The increase in carbonyl compounds (Figure 2) reflects a situation of dicarbonyl stress, that can modify function and structure of biomolecules, increasing the ROS (reactive oxygen species) and consequently the oxidative stress.[44] This unbalance of ROS and carbonyl compounds can lead an increase in protein changes and contributing to cell and tissue dysfunction.[23–25,29] This rise in carbonyl compounds reinforce our hypothesis that diacetyl intake cooperates to rise the oxidative stress and can lead to protein modifications. Besides, when dicarbonyl concentrations get rise, also increase the potential for protein and cell dysfunction leading to the organisms developed a disease or impaired health.[29]

As expected, we can see in figure 3a, through the heatmap, the clear separation of the control group from the two groups treated with diacetyl. There is some expressive difference between groups as show in figure 3b, seasonable we can differentiate perfectly of three groups, so when we add PC1 and PC2 we get 100% separation. This result expressed by the figures 3a and 3b shows that diacetyl intake in both dosages (200 and 300 mg kg^{-1} day^{-1}) cause changes in the metabolism of this animals causing harm to health.

The animals treated with diacetyl also suffered changes in the protein plasmatic profile (Table II), we observe the decrease in albumin. This protein (Alb) is the most abundant protein in the plasma and perform important role to transport of substances in organism. Moreover, the albumin is related with indirect detoxification of extracellular MGO, because this way MGO can cross the cell membrane and be detoxified by glyoxalase system.[43,44] Besides MGO has fast and strong MG scavenging effect of albumin[43] and this scavenging effect can modify this protein and thus not allow or hinder its identification. Beyond albumin we observed the protein A2M that presented quantitative decrease in treated group with 300 mg kg^{-1} day^{-1} of diacetyl. The protein (A2M) is an antiprotease involved in the blood coagulation and the low expression of this protein contributing to the allergic inflammatory response[45,46] and had a significant negative correlation with age.[47] Already the proteins A1AT4 and HA10 were upregulated, the A1AT4 is a serine proteinase inhibitor that are involved in proteolytic processes regulation including fibrinolysis and inflammation.[48,49] The protein HA10 as describe in uniport is involved in the presentation of foreign antigens to the immune system and as Li *et. al,*[50] A1AT4 contribute to the augmented activation of T lymphocytes. How we show in Figure 4 this modification in proteins promotes changes in some metabolic and biologic process as well promotes alterations in important pathways especially the T-cell activation and blood coagulation consequently leads to break of homeostasis and may induce disease development.

CONCLUSIONS

These works suggest that the consumption of diacetyl is harmful and capable of promoting changes in the redox balance. Once, diacetyl intake decreases levels of MGO in liver tissue and increase carbonyl species in plasma moreover promoted alteration in proteomic plasmatic profile in mice and consequently in the metabolic profile. The data indicate that carbonyl stress can represent an important mechanism to alteration the protein profile probably caused by diacetyl intake.

The results from here open a gap to be explored in future research on the likely detoxification of MGO when high concentrations of diacetyl have been ingested.

Conflicts of interest

The authors declare that there is no conflict of interest in the preparation of this article.

Acknowledgements

We would like to acknowledge the *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior*-CAPES [grant number 2012/02514-9], the Brazilian Innovation Agency-FINEP [grant number 2013/07763-0], and the FAPESP São Paulo Research Foundation (FAPESP) [grant number 2010/01404- 0].

REFERENCES

(1) Papetti, A.; Mascherpa, D.; Gazzani, G. Free α-dicarbonyl compounds in coffee, barley coffee and soy sauce and effects of *in vitro* digestion. *Food Chem.* **2014**, *164*, 259-265. https://doi.org/10.1016/j.foodchem.2014.05.022

(2) Cavalcanti, Z. R.; Filho, A. P. L. A.; Pereira, C. A. C.; Coletta, E. N. A. M. Bronchiolitis associated with exposure to artificial butter flavoring in workers at a cookie factory in Brazil. *Jornal Brasileiro de Pneumologia* **2012**, *38* (3). https://doi.org/10.1590/S1806-37132012000300016

(3) Bartowsky, E. J.; Henschke, P. A. The “buttery” attribute of wine - diacetyl - desirability, spoilage and beyond. *Int. J. Food Microbiol.* **2004**, *96* (3), 235-252. https://doi.org/10.1016/j.ijfoodmicro.2004.05.013

(4) Bortoleto, G. G.; Gomes, W. P. C.; Ushimura, L. C.; Bonança, R. A.; Novello, E. H. Evaluation of the profile of volatile organic compounds in industrial and craft beers. *J. Microbiol., Biotechnol. Food Sci.* **2022**, *12* (2), e5532. https://doi.org/10.55251/jmbfs.5532
(5) Gaffney, S. H.; Abelmann, A.; Pierce, J. S.; Glynn, M. E.; Henshaw, J. L.; McCarthy, L. A.; Lotter, J. T.; Liong, M.; Finley, B. L. Naturally occurring diacetyl and 2,3-pentanedione concentrations associated with roasting and grinding unflavored coffee beans in a commercial setting. *Toxico. Rep.* **2015**, *2*, 1171-1181. https://doi.org/10.1016/j.toxrep.2015.08.003
(6) National Center for Biotechnology Information. *PubChem Compound Summary for CID 650, 2,3-Butanedione*. https://pubchem.ncbi.nlm.nih.gov/compound/2_3-butanedione [accessed: February, 2024].
(7) Rincon-Delgadillo, M. I.; Lopez-Hernandez, A.; Wijaya, I.; Rankin, S. A. Diacetyl levels and volatile profiles of commercial starter distillates and selected dairy foods. *J. Dairy Sci.* **2012**, *95* (3), 1128-1139. https://doi.org/10.3168/jds.2011-4834
(8) Shibamoto, T. Diacetyl: Occurrence, Analysis, and Toxicity. *J. Agric. Food Chem.* **2014**, *62* (18), 4048-4053. https://doi.org/10.1021/jf500615u
(9) Allen, J. G.; Flanigan, S. S.; LeBlanc, M.; Vallarino, J.; MacNaughton, P.; Stewart, J. H.; Christiani, D. C. Flavoring Chemicals in E-Cigarettes: Diacetyl, 2,3-Pentanedione, and Acetoin in a Sample of 51 Products, Including Fruit-, Candy-, and Cocktail-Flavored E-Cigarettes. *Environ. Health Perspect.* **2016**, *124* (6), 733-739. https://doi.org/10.1289/ehp.1510185
(10) Pierce, J. S.; Abelmann, A.; Spicer, L. J.; Adams, R. E.; Finley, B. L. Diacetyl and 2,3-pentanedione exposures associated with cigarette smoking: implications for risk assessment of food and flavoring workers. *Crit. Rev. Toxicol.* **2014**, *44* (5), 420-435. https://doi.org/10.3109/10408444.2014.882292
(11) Alves, A. N. L.; Jedlicka, L. D. L.; Massari, J.; Juliano, M. A.; Bechara, E. J. H.; Assunção, N. A. Electrospray Ionization Mass Spectrometry Applied to Study the Radical Acetylation of Amino Acids, Peptides and Proteins. *J. Braz. Chem. Soc.* **2013**, *24* (12), 1983-1990. https://doi.org/10.5935/0103-5053.20130248
(12) Massari, J.; Fujiy, D. E.; Dutra, F.; Vaz, S. M.; Costa, A. C. O.; Micke, G. A.; Tavares, M. F. M.; Tokikawa, R.; Assunção, N. A.; Bechara, E. J. H. Radical Acetylation of 2′-Deoxyguanosine and L-Histidine Coupled to the Reaction of Diacetyl with Peroxynitrite in Aerated Medium. *Chem. Res. Toxicol.* **2008**, *21* (4), 879-887. https://doi.org/10.1021/tx7002799
(13) Massari, J.; Tokikawa, R.; Medinas, D. B.; Angeli, J. P. F.; Di Mascio, P.; Assunção, N. A.; Bechara, E. J. H. Generation of Singlet Oxygen by the Glyoxal-Peroxynitrite System. *J. Am. Chem. Soc.* **2011**, *133* (51), 20761-20768. https://doi.org/10.1021/ja2051414
(14) Massari, J.; Tokikawa, R.; Zanolli, L.; Tavares, M. F. M.; Assunção, N. A.; Bechara, E. J. H. Acetyl Radical Production by the Methylglyoxal-Peroxynitrite System: A Possible Route for l-Lysine Acetylation. *Chem. Res. Toxicol.* **2010**, *23* (11), 1762-1770. https://doi.org/10.1021/tx1002244
(15) Tokikawa, R.; Loffredo, C.; Uemi, M.; Machini, M. T.; Bechara, E. J. H. Radical Acylation of L-Lysine Derivatives and L-Lysine-Containing Peptides by Peroxynitrite-Treated Diacetyl and Methylglyoxal. *Free Radic. Res.* **2014**, *48* (3), 357-370. https://doi.org/10.3109/10715762.2013.871386
(16) Jedlicka, L. D. L.; Guterres, S. B.; Balbino, A. M.; Neto, G. B.; Landgraf, R. G.; Fernandes, L.; Carrilho, E.; Bechara, E. J. H.; Assuncao, N. A. Increased Chemical Acetylation of Peptides and Proteins in Rats after Daily Ingestion of Diacetyl Analyzed by Nano-LC-MS/MS. *PeerJ* 6:e4688 **2018**. https://doi.org/10.7717/peerj.4688
(17) Jedlicka, L. D. L.; Silva, J. D. C.; Balbino, A. M.; Neto, G. B.; Furtado, D. Z. S.; Silva, H. D. T. Da; Cavalcanti, F. D. B. C.; Van Der Heijden, K. M.; Penatti, C. A. A.; Bechara, E. J. H.; Assunção, N. A. Effects of Diacetyl Flavoring Exposure in Mice Metabolism. *Biomed Res. Int.* **2018**, *2018*, Article ID 9875319. https://doi.org/10.1155/2018/9875319
(18) Espinosa-Diez, C.; Miguel, V.; Mennerich, D.; Kietzmann, T.; Sánchez-Pérez, P.; Cadenas, S.; Lamas, S. Antioxidant Responses and Cellular Adjustments to Oxidative Stress. *Redox Biology* **2015**, *6*, 183-197. https://doi.org/10.1016/j.redox.2015.07.008

(19) Schafer, F. Q.; Buettner, G. R. Redox Environment of the Cell as Viewed through the Redox State of the Glutathione Disulfide/Glutathione Couple. *Free Radical Biol. Med*. **2001**, *30* (11), 1191-1212. https://doi.org/10.1016/S0891-5849(01)00480-4

(20) Silva, M. S.; Gomes, R. A.; Ferreira, A. E. N.; Freire, A. P.; Cordeiro, C. The glyoxalase pathway: the first hundred years... and beyond. *Biochem. J*. **2013**, *453* (1), 1-15. https://doi.org/10.1042/BJ20121743

(21) Dutra, F.; Bechara, E. J. H. Bioquímica e Ação Citotóxica de Alfa-Aminocetonas Endógenas. *Quim. Nova* **2005**, *28* (3). https://doi.org/10.1590/s0100-40422005000300021

(22) Mika, D.; Guruvayoorappan, C. Experimental study on anti-tumor and anti-inflammatory effect of *Thespesia populnea* phytochemical extract in mice models. *Immunopharmacol. Immunotoxicol.* **2013**, *35* (1), 157-163. https://doi.org/10.3109/08923973.2012.735237

(23) Rabbani, N.; Thornalley, P. J. Measurement of Methylglyoxal by Stable Isotopic Dilution Analysis LC-MS/MS with Corroborative Prediction in Physiological Samples. *Nat. Protoc.* **2014**, *9* (8), 1969-1979. https://doi.org/10.1038/nprot.2014.129

(24) Tatone, C.; Heizenrieder, T.; Di Emidio, G.; Treffon, P.; Amicarelli, F.; Seidel, T.; Eichenlaub-Ritter, U. Evidence that carbonyl stress by methylglyoxal exposure induces DNA damage and spindle aberrations, affects mitochondrial integrity in mammalian oocytes and contributes to oocyte ageing. *Hum. Reprod.* **2011**, *26* (7), 1843-1859. https://doi.org/10.1093/humrep/der140

(25) Donato, L.; Scimone, C.; Alibrandi, S.; Nicocia, G.; Rinaldi, C.; Sidoti, A.; D'Angelo, R. Discovery of *GLO1* New Related Genes and Pathways by RNA-Seq on A2E-Stressed Retinal Epithelial Cells Could Improve Knowledge on Retinitis Pigmentosa. *Antioxidants* **2020**, *9* (5), 416. https://doi.org/10.3390/antiox9050416

(26) Lovric, J. *Introducing Proteomics: From Concepts to Sample Separation, Mass Spectrometry and Data Analysis*. John Wiley & Sons, 2011.

(27) Zucker, I. The Mixed Legacy of the Rat Estrous Cycle. *Biol. Sex. Differ.* **2023**, *14*, Article number: 55. https://doi.org/10.1186/s13293-023-00542-7

(28) Colley, J.; Gaunt, I. F.; Lansdown, A. B. G.; Grasso, P.; Gangolli, S. D. Acute and short-term toxicity of diacetyl in rats. *Food Cosmet. Toxicol.* **1969**, *7*, 571-580. https://doi.org/10.1016/s0015-6264(69)80460-8

(29) Rabbani, N.; Xue, M.; Thornalley, P. J. Dicarbonyls and glyoxalase in disease mechanisms and clinical therapeutics. *Glycoconj. J.* **2016**, *33*, 513-525. https://doi.org/10.1007/s10719-016-9705-z

(30) Levine, R. L.; Garland, D.; Oliver, C. N.; Amici, A.; Climent, I.; Lenz, A. G.; Ahn, B. W.; Shaltiel, S.; Stadtman, E. R. Determination of carbonyl content in oxidatively modified proteins. *Methods Enzymol.* **1990**, *186*, 464-478. https://doi.org/10.1016/0076-6879(90)86141-H

(31) Bradford, M. M. A Rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **1976**, *72* (1–2), 248-254. https://doi.org/10.1016/0003-2697(76)90527-3

(32) The UniProt Consortium. UniProt: The Universal Protein Knowledgebase in 2023. *Nucleic Acids Res.* **2023**, *51* (D1), D523-D531. https://doi.org/10.1093/nar/gkac1052

(33) Bantscheff, M.; Lemeer, S.; Savitski, M. M.; Kuster, B. Quantitative Mass Spectrometry in Proteomics: Critical Review Update from 2007 to the Present. *Anal. Bioanal. Chem.* **2012**, *404*, 939-965. https://doi.org/10.1007/s00216-012-6203-4

(34) Silva, J. C.; Gorenstein, M. V.; Li, G. Z.; Vissers, J. P. C.; Geromanos, S. J. Absolute Quantification of Proteins by LCMSE: A Virtue of Parallel MS Acquisition. *Mol. Cell. Proteomics* **2006**, *5* (1), 144-156. https://doi.org/10.1074/mcp.M500230-MCP200

(35) Thomas, P. D.; Ebert, D.; Muruganujan, A.; Mushayahama, T.; Albou, L. P.; Mi, H. PANTHER: Making genome-scale phylogenetics accessible to all. *Protein Sci*. **2022**, *31* (1), 8-22. https://doi.org/10.1002/pro.4218

(36) The UniProt Consortium. *Taxonomy - Mus musculus*. https://www.uniprot.org/taxonomy/10090
(37) Metsalu, T.; Vilo, J. ClustVis: a web tool for visualizing clustering of multivariate data using Principal Component Analysis and heatmap. *Nucleic Acids Res.* **2015**, *43* (W1), W566-W570. https://doi.org/10.1093/nar/gkv468
(38) Forman, H. J.; Zhang, H.; Rinna, A. Glutathione: Overview of its protective roles, measurement, and biosynthesis. *Mol. Aspects Med.* **2009**, *30* (1-2), 1-12. https://doi.org/10.1016/j.mam.2008.08.006.
(39) Julius, M.; Lang, C. A.; Gleiberman, L.; Harburg, E.; Difranceisco, W.; Schork, A. Glutathione and morbidity in a community-based sample of elderly. *J. Clin. Epidemiol.* **1994**, *47* (9), 1021-1026. https://doi.org/10.1016/0895-4356(94)90117-1
(40) Streck, E. L.; De Prá, S. D. T.; Ferro, P. R.; Carvalho-Silva, M.; Gomes, L. M.; Agostini, J. F.; Damiani, A.; Andrade, V. M.; Schuck, P. F.; Ferreira, G. C.; Scaini, G. Role of antioxidant treatment on DNA and lipid damage in the brain of rats subjected to a chemically induced chronic model of tyrosinemia type II. *Mol. Cell. Biochem.* **2017**, *435*, 207-214. https://doi.org/10.1007/s11010-017-3070-5
(41) Schmitz, A. E.; de Souza, L. F.; dos Santos, B.; Maher, P.; Lopes, F. M.; Londero, G. F.; Klamt, F.; Dafre, A. L. Methylglyoxal-Induced Protection Response and Toxicity: Role of Glutathione Reductase and Thioredoxin Systems. *Neurotox. Res.* **2017**, *32*, 340-350. https://doi.org/10.1007/s12640-017-9738-5
(42) Shangari, N.; Depeint, F.; Furrer, R.; Bruce, W. R.; Popovic, M.; Zheng, F.; O'Brien, P. J. A thermolyzed diet increases oxidative stress, plasma α-aldehydes and colonic inflammation in the rat. *Chem.-Biol. Interact.* **2007**, *169* (2), 100-109. https://doi.org/10.1016/j.cbi.2007.05.009
(43) Zunkel, K.; Simm, A.; Bartling, B. Long-Term Intake of the Reactive Metabolite Methylglyoxal Is Not Toxic in Mice. *Chem. Toxicol.* **2020**, *141*, 111333. https://doi.org/10.1016/j.fct.2020.111333
(44) Kalapos, M. P. Where does plasma methylglyoxal originate from? *Diabetes Res. Clin. Pract.* **2013**, *99* (3), 260-271. https://doi.org/10.1016/j.diabres.2012.11.003
(45) Chen, X.; Xie, Z. H.; Lv, Y. X.; Tang, Q. P.; Zhang, H.; Zhang, J. Y.; Wu, B.; Jiang, W. H. A Proteomics Analysis Reveals That A2M Might Be Regulated by STAT3 in Persistent Allergic Rhinitis. *Clin. Exp. Allergy* **2016**, *46* (6), 813-824. https://doi.org/10.1111/cea.12711
(46) Barbosa, J. H. P.; Souza, I. T.; Santana, A. E. G.; Goulart, M. O. F. A determinação dos produtos avançados de glicação (AGEs) e de lipoxidação (ALEs) em alimentos e em sistemas biológicos: Avanços, Desafios e Perspectivas. *Quim. Nova* **2016**, *39* (5), 608-620. http://dx.doi.org/10.5935/0100-4042.20160048
(47) Rugsarash, W.; Tungtrongchitr, R.; Petmitr, S.; Phonrat, B.; Pongpaew, P.; Harnroongroj, T.; Tungtrongchitr, A. The genetic association between alpha-2-macroglobulin (A2M) gene deletion polymorphism and low serum A2M concentration in overweight/obese Thais. *Nutr. Neurosci.* **2006**, *9* (1–2), 93-98. https://doi.org/10.1080/10284150600771777
(48) Barbour, K. W.; Goodwin, R. L.; Guillonneau, F.; Wang, Y.; Baumann, H.; Berger, F. G. Functional Diversification during Evolution of the Murine A1-Proteinase Inhibitor Family: Role of the Hypervariable Reactive Center Loop. *Mol. Biol. Evol.* **2002**, *19* (5), 718-727. https://doi.org/10.1093/oxfordjournals.molbev.a004130
(49) Wright, H. T. The Structural Puzzle of How Serpin Serine Proteinase Inhibitors Work. *BioEssays* **1996**, *18* (6). https://doi.org/10.1002/bies.950180607
(50) Li, D. A.; He, Y.; Guo, Y. J.; Wang, F.; Song, S. X.; Wang, Y.; Yang, F.; He, X. W.; Sun, S. H. Comparative Proteomics Analysis to Annexin B1 DNA and Protein Vaccination in Mice. *Vaccine* **2007**, *25* (5), 932-938. https://doi.org/10.1016/j.vaccine.2006.08.042

SUPPLEMENTARY MATERIAL

Table S1. Parameters for validation of the separation method of GSH and GSSG

Compounds	LOD (nmol L^{-1})	LOQ (nmol L^{-1})	Linearity (nmol L^{-1})	Equation	r^2	Lack of fit (p<0,05)
GSH	0.16	0.40	79.52 - 2544.79	y = 34100.78+1396.1x	0.995	0.754
GSSG	0.07	0.27	79.78 - 618.33	y = -56696.30+4599.7x	0.995	0.191

Table S2. Instrumental accuracy of the GSH and GSSG separation in standards and sample along with recovery values for the extraction method

Precision	Type	Levels*	GSH	GSSG
Intra-day (%, n=10)	Standard	N1	10.32	11.23
		N2	3.25	7.87
		N3	2.57	4.56
	Sample	N1	13.15	11.67
		N2	11.82	9.25
		N3	8.33	10.78
Intra-day (%, n=5)	Standard	N1	11.85	11.90
		N2	5.08	10.51
		N3	2.44	9.66
	Sample	N1	11.58	13.12
		N2	9.67	11.37
		N3	8.48	9.98
Recovery (%, mean ± DP, n = 4)[c]		N1	98.69±8.95	97.94±5.67
		N2	101.11±6.38	101.13±4.73
		N3	100.48±4.35	100.47±8.53

*Concentration of standards: N1: 79 nmol L^{-1} of GSH; 79 nmol L^{-1} of GSSG; N2: 1204 nmol L^{-1} of GSH; 349 nmol L^{-1} of GSSG; N3: 2544 nmol L^{-1} of GSH; 618 nmol L^{-1} GSSG.

Table S3. Summary of the ANOVA of the linear model used in the quantification of methylglyoxal

Source of Variance	QS	d.f.	MS	Regression		Lack of fit	
				MS_R/MS_r	$F_{1,14,95\%}$	MS_{Lad}/MS_{Pe}	$F_{4,12,95\%}$
Regression	$2.23x10^{12}$	1	$2.23x10^{12}$	3408.01	4.60	0.862	3.26
Lack of fit	$2.25x10^{9}$	4	$5.64x10^{8}$				
Pure Error	$7.85x10^{9}$	12	$6.54x10^{8}$				
QS_{Total}	$2.24x10^{12}$	17					

QS: quadratic sum; d.f.: degrees of freedom; MS_R: regression mean square, MS_r: residue mean.

Table S4. Variance explained by principal components (3 components)

	PC1	PC2	PC3
Individual	0.81	0.19	0.00
Cumulative	0.81	1.00	1.00

Table S5. Principal components (3 data points in rows, 3 components in columns)

	PC1	PC2	PC3
Control	-2.32	-0.09	0.00
200 mg	1.31	-0.93	-0.00
300 mg	1.00	1.02	0.00

Table S6. Component loadings (5 dimensions in rows, 3 components in columns)

	PC1	PC2	PC3
GSH	0.43	-0.52	0.25
GSSG	-0.49	-0.13	0.85
GSH/GSSG	0.48	-0.23	0.20
MGO	-0.50	-0.05	-0.23
Carbonyl stress	0.30	0.81	0.34

Table S7. Identified proteins in control group (three technical replicates)

Replicate number	Protein Description	Protein Matched Products	Protein matched Peptides	Protein seq Cover (%)
1	Serum albumin OS=Mus musculus GN=Alb PE=1 SV=3	769	58	65.46
1	Apolipoprotein A-I OS=Mus musculus GN=Apoa1 PE=1 SV=2	194	18	38.26
1	Hemoglobin subunit alpha OS=Mus musculus GN=Hba PE=1 SV=2	70	6	52.82
1	Serotransferrin OS=Mus musculus GN=Tf PE=1 SV=1	541	50	54.52
1	Serine protease inhibitor A3K OS=Mus musculus GN=Serpina3k PE=1 SV=2	238	18	31.58
1	Alpha-2-macroglobulin OS=Mus musculus GN=A2m PE=1 SV=3	699	62	33.71
1	Alpha-1-antitrypsin 1-5 OS=Mus musculus GN=Serpina1e PE=1 SV=1	144	16	34.87
1	Alpha-1-antitrypsin 1-4 OS=Mus musculus GN=Serpina1d PE=2 SV=1	236	24	42.37
1	Carboxylesterase 1C OS=Mus musculus GN=Ces1c PE=1 SV=4	170	18	33.57
1	Alpha-1-antitrypsin 1-3 OS=Mus musculus GN=Serpina1c PE=1 SV=2	201	20	43.2

(continued on next page)

Table S7. Identified proteins in control group (three technical replicates) (continued)

Replicate number	Protein Description	Protein Matched Products	Protein matched Peptides	Protein seq Cover (%)
1	Alpha-1-antitrypsin 1-1 OS=Mus musculus GN=Serpina1a PE=1 SV=4	201	20	43.1
1	Alpha-1-antitrypsin 1-2 OS=Mus musculus GN=Serpina1b PE=1 SV=2	191	17	36.08
1	Serine protease inhibitor A3M OS=Mus musculus GN=Serpina3m PE=1 SV=2	92	9	16.27
1	Hemoglobin subunit beta-1 OS=Mus musculus GN=Hbb-b1 PE=1 SV=2	39	6	47.62
1	Hemopexin OS=Mus musculus GN=Hpx PE=1 SV=2	140	18	50.22
1	Kininogen-1 OS=Mus musculus GN=Kng1 PE=1 SV=1	100	13	29.05
1	Enolase 1 OS=Saccharomyces cerevisiae (st	119	16	43.48
1	Isoform 3 of Kininogen-1 OS=Mus musculus GN=Kng1	94	12	36.25
1	Isoform LMW of Kininogen-1 OS=Mus musculus GN=Kng1	94	12	40.28
1	Hemoglobin subunit beta-2 OS=Mus musculus GN=Hbb-b2 PE=1 SV=2	28	4	23.81
1	Complement C3 OS=Mus musculus GN=C3 PE=1 SV=3	248	37	25.62
1	Hemoglobin subunit epsilon-Y2 OS=Mus musculus GN=Hbb-y PE=1 SV=2	19	2	6.8
1	Murinoglobulin-1 OS=Mus musculus GN=Mug1 PE=1 SV=3	234	28	19.44
1	Apolipoprotein A-II OS=Mus musculus GN=Apoa2 PE=1 SV=2	40	5	53.92
1	Carboxylesterase 1D OS=Mus musculus GN=Ces1d PE=1 SV=1	34	4	9.2
1	Serine protease inhibitor A3G OS=Mus musculus GN=Serpina3g PE=2 SV=2	37	8	21.82
1	Serine protease inhibitor A3C OS=Mus musculus GN=Serpina3c PE=2 SV=1	33	5	6.47
1	Serine protease inhibitor A3N OS=Mus musculus GN=Serpina3n PE=1 SV=1	33	5	6.46
1	Serine protease inhibitor A3F OS=Mus musculus GN=Serpina3f PE=1 SV=3	34	6	16.18
1	Beta-2-glycoprotein 1 OS=Mus musculus GN=Apoh PE=1 SV=1	60	11	31.59
1	Alpha-2-HS-glycoprotein OS=Mus musculus GN=Ahsg PE=1 SV=1	44	6	26.67
1	Liver carboxylesterase 1 OS=Mus musculus GN=Ces1 PE=2 SV=1	23	2	4.07
1	Ig heavy chain V region AC38 205.12 OS=Mus musculus PE=1 SV=1	11	1	16.1
1	Ig heavy chain V region J558 OS=Mus musculus PE=1 SV=1	11	1	16.24
1	Ig heavy chain V region MOPC 104E OS=Mus musculus PE=1 SV=1	11	1	16.24
1	Murinoglobulin-2 OS=Mus musculus GN=Mug2 PE=2 SV=2	146	18	10.96
1	Apolipoprotein A-IV OS=Mus musculus GN=Apoa4 PE=1 SV=3	60	11	27.34

(continued on next page)

Table S7. Identified proteins in control group (three technical replicates) (continued)

Replicate number	Protein Description	Protein Matched Products	Protein matched Peptides	Protein seq Cover (%)
1	Isoform 2 of Ig mu chain C region OS=Mus musculus GN=Ighm	80	12	28.21
1	Ig mu chain C region OS=Mus musculus GN=Ighm PE=1 SV=2	80	12	29.52
1	Ig kappa chain V-III region MOPC 70 OS=Mus musculus PE=1 SV=1	18	2	30.63
1	Ig kappa chain V-III region PC 7132 OS=Mus musculus PE=1 SV=1	18	2	30.36
1	Ig kappa chain V-III region 50S10.1 OS=Mus musculus PE=1 SV=1	18	2	30.63
1	Ig kappa chain V-III region PC 2880/PC 1229 OS=Mus musculus PE=1 SV=1	18	2	30.63
1	Vitamin D-binding protein OS=Mus musculus GN=Gc PE=1 SV=2	98	16	30.67
1	Ig kappa chain V-III region PC 6684 OS=Mus musculus PE=1 SV=1	14	1	16.22
1	Ig kappa chain V-III region PC 7769 OS=Mus musculus PE=1 SV=1	14	1	16.22
1	Ig kappa chain V-III region PC 7175 OS=Mus musculus PE=1 SV=1	14	1	16.22
1	Ig kappa chain V-III region PC 2485/PC 4039 OS=Mus musculus PE=1 SV=1	14	1	16.22
1	Ig kappa chain V-III region PC 7940 OS=Mus musculus PE=1 SV=1	14	1	16.22
1	Ig kappa chain V-III region PC 7183 OS=Mus musculus PE=1 SV=1	14	1	16.22
1	Ig kappa chain V-III region PC 7210 OS=Mus musculus PE=1 SV=1	14	1	16.36
1	Ig kappa chain V-III region PC 6308 OS=Mus musculus PE=1 SV=1	14	1	16.22
1	Ig kappa chain V-III region PC 7043 OS=Mus musculus PE=1 SV=1	14	1	16.22
1	Ig kappa chain V-III region CBPC 101 OS=Mus musculus PE=1 SV=1	14	1	16.22
1	Ig kappa chain V-III region PC 3741/TEPC 111 OS=Mus musculus PE=1 SV=1	14	1	16.22
1	Ig kappa chain V-III region TEPC 124 OS=Mus musculus PE=1 SV=1	14	1	16.07
1	Ig kappa chain V-III region PC 2413 OS=Mus musculus PE=1 SV=1	14	1	16.22
1	Clusterin OS=Mus musculus GN=Clu PE=1 SV=1	52	9	26.34
1	Isoform Short of Complement C3 OS=Mus musculus GN=C3	39	7	15.14
1	Apolipoprotein E OS=Mus musculus GN=Apoe PE=1 SV=2	29	5	18.97
1	Complement factor B OS=Mus musculus GN=Cfb PE=1 SV=2	75	12	15.24
1	Fibrinogen alpha chain OS=Mus musculus GN=Fga PE=2 SV=1	61	14	25.73
1	Isoform 2 of Fibrinogen alpha chain OS=Mus musculus GN=Fga	60	13	32.68
1	Corticosteroid-binding globulin OS=Mus musculus GN=Serpina6 PE=1 SV=1	23	4	9.07
1	Ceruloplasmin OS=Mus musculus GN=Cp PE=1 SV=2	109	17	21.39

(continued on next page)

Table S7. Identified proteins in control group (three technical replicates) (continued)

Replicate number	Protein Description	Protein Matched Products	Protein matched Peptides	Protein seq Cover (%)
1	Serum paraoxonase/arylesterase 1 OS=Mus musculus GN=Pon1 PE=1 SV=2	14	3	15.21
1	Beta-2-microglobulin OS=Mus musculus GN=B2m PE=1 SV=2	9	2	14.29
1	Alpha-2-antiplasmin OS=Mus musculus GN=Serpinf2 PE=1 SV=1	35	6	10.59
1	Histidine-rich glycoprotein OS=Mus musculus GN=Hrg PE=1 SV=2	43	10	18.48
1	Fibrinogen gamma chain OS=Mus musculus GN=Fgg PE=1 SV=1	36	7	14.45
1	Complement factor H OS=Mus musculus GN=Cfh PE=1 SV=2	57	11	15.4
1	Plasminogen OS=Mus musculus GN=Plg PE=1 SV=3	72	14	15.52
1	Isoform 2 of Ig gamma-2B chain C region OS=Mus musculus GN=Igh-3	20	5	13.43
1	Ig gamma-2B chain C region OS=Mus musculus GN=Igh-3 PE=1 SV=3	20	5	11.14
1	Mannose-binding protein C OS=Mus musculus GN=Mbl2 PE=2 SV=2	14	2	11.89
1	Prothrombin OS=Mus musculus GN=F2 PE=1 SV=1	38	7	14.72
1	Plasma protease C1 inhibitor OS=Mus musculus GN=Serping1 PE=1 SV=3	28	5	16.67
1	Gelsolin OS=Mus musculus GN=Gsn PE=1 SV=3	52	10	13.08
1	Isoform 2 of Gelsolin OS=Mus musculus GN=Gsn	48	9	13
1	Leucine zipper protein 2 OS=Mus musculus GN=Luzp2 PE=2 SV=1	25	7	33.04
1	Apolipoprotein C-III OS=Mus musculus GN=Apoc3 PE=1 SV=2	3	1	19.19
1	Reversed Sequence 25008	5	1	4.76
1	Lactotransferrin OS=Mus musculus GN=Ltf PE=2 SV=4	16	5	9.05
1	Antithrombin-III OS=Mus musculus GN=Serpinc1 PE=1 SV=1	61	13	28.17
1	Alpha-2-macroglobulin-P OS=Mus musculus GN=A2mp PE=2 SV=2	36	12	8.89
1	Inter-alpha-trypsin inhibitor heavy chain H2 OS=Mus musculus GN=Itih2 PE=1 SV=1	53	9	11.63
1	Heparin cofactor 2 OS=Mus musculus GN=Serpind1 PE=1 SV=1	18	5	11.09
1	Carboxypeptidase N subunit 2 OS=Mus musculus GN=Cpn2 PE=1 SV=2	17	3	5.3
1	Reversed Sequence 12561	14	3	13.39
1	Fibrinogen beta chain OS=Mus musculus GN=Fgb PE=2 SV=1	48	10	25.16
1	Succinate dehydrogenase [ubiquinone] cytochrome b small subunit_ mitochondrial OS=Mus musculus GN=Sdhd PE=2 SV=2	9	2	15.09
1	Reversed Sequence 25034	13	4	15.73
1	Isoform 2 of Alpha-(1_3)-fucosyltransferase 10 OS=Mus musculus GN=Fut10	15	4	17.89

(continued on next page)

Table S7. Identified proteins in control group (three technical replicates) (continued)

Replicate number	Protein Description	Protein Matched Products	Protein matched Peptides	Protein seq Cover (%)
1	Alpha-(1_3)-fucosyltransferase 10 OS=Mus musculus GN=Fut10 PE=2 SV=1	16	5	19.33
1	Fetuin-B OS=Mus musculus GN=Fetub PE=1 SV=1	14	2	4.9
1	Reversed Sequence 1705	16	4	43.15
1	Transthyretin OS=Mus musculus GN=Ttr PE=1 SV=1	10	2	19.05
1	Complement component C9 OS=Mus musculus GN=C9 PE=1 SV=2	18	6	11.5
1	Ig alpha chain C region OS=Mus musculus PE=1 SV=1	9	1	4.36
1	Isocitrate dehydrogenase [NADP] cytoplasmic OS=Mus musculus GN=Idh1 PE=1 SV=2	17	3	7.25
1	Isoform 3 of Afamin OS=Mus musculus GN=Afm	17	8	15.06
1	Afamin OS=Mus musculus GN=Afm PE=1 SV=2	17	8	15.13
1	Plasma kallikrein OS=Mus musculus GN=Klkb1 PE=1 SV=2	24	7	13.48
1	Zinc-alpha-2-glycoprotein OS=Mus musculus GN=Azgp1 PE=1 SV=2	12	4	12.05
1	Phosphatidylinositol-glycan-specific phospholipase D OS=Mus musculus GN=Gpld1 PE=1 SV=1	38	8	10.63
1	Isoform 4 of DENN domain-containing protein 1B OS=Mus musculus GN=Dennd1b	6	2	9.22
1	Isoform 2 of DENN domain-containing protein 1B OS=Mus musculus GN=Dennd1b	8	3	8.54
1	Proline-rich transmembrane protein 4 OS=Mus musculus GN=Prrt4 PE=2 SV=3	8	2	2.88
2	Serum albumin OS=Mus musculus GN=Alb PE=1 SV=3	793	65	70.23
2	Apolipoprotein A-I OS=Mus musculus GN=Apoa1 PE=1 SV=2	192	19	37.12
2	Serine protease inhibitor A3K OS=Mus musculus GN=Serpina3k PE=1 SV=2	213	16	31.34
2	Alpha-2-macroglobulin OS=Mus musculus GN=A2m PE=1 SV=3	698	63	37.53
2	Hemoglobin subunit alpha OS=Mus musculus GN=Hba PE=1 SV=2	69	5	54.23
2	Alpha-1-antitrypsin 1-4 OS=Mus musculus GN=Serpina1d PE=2 SV=1	215	20	41.16
2	Serotransferrin OS=Mus musculus GN=Tf PE=1 SV=1	549	51	55.81
2	Alpha-1-antitrypsin 1-3 OS=Mus musculus GN=Serpina1c PE=1 SV=2	187	17	39.08
2	Alpha-1-antitrypsin 1-1 OS=Mus musculus GN=Serpina1a PE=1 SV=4	187	17	38.98
2	Alpha-1-antitrypsin 1-2 OS=Mus musculus GN=Serpina1b PE=1 SV=2	198	17	38.98
2	Serine protease inhibitor A3M OS=Mus musculus GN=Serpina3m PE=1 SV=2	96	9	16.27

(continued on next page)

Table S7. Identified proteins in control group (three technical replicates) (continued)

Replicate number	Protein Description	Protein Matched Products	Protein matched Peptides	Protein seq Cover (%)
2	Alpha-1-antitrypsin 1-5 OS=Mus musculus GN=Serpina1e PE=1 SV=1	125	13	34.87
2	Hemoglobin subunit beta-1 OS=Mus musculus GN=Hbb-b1 PE=1 SV=2	61	8	73.47
2	Hemoglobin subunit beta-2 OS=Mus musculus GN=Hbb-b2 PE=1 SV=2	41	4	21.77
2	Carboxylesterase 1C OS=Mus musculus GN=Ces1c PE=1 SV=4	168	17	34.84
2	Enolase 1 OS=Saccharomyces cerevisiae (st	132	18	45.77
2	Complement C3 OS=Mus musculus GN=C3 PE=1 SV=3	234	36	23.99
2	Hemoglobin subunit epsilon-Y2 OS=Mus musculus GN=Hbb-y PE=1 SV=2	21	2	6.8
2	Hemopexin OS=Mus musculus GN=Hpx PE=1 SV=2	171	23	59.13
2	Beta-2-glycoprotein 1 OS=Mus musculus GN=Apoh PE=1 SV=1	57	10	37.68
2	Murinoglobulin-1 OS=Mus musculus GN=Mug1 PE=1 SV=3	232	29	20.05
2	Kininogen-1 OS=Mus musculus GN=Kng1 PE=1 SV=1	87	13	24.81
2	Isoform 3 of Kininogen-1 OS=Mus musculus GN=Kng1	80	12	30.42
2	Isoform LMW of Kininogen-1 OS=Mus musculus GN=Kng1	80	12	33.8
2	Alpha-2-HS-glycoprotein OS=Mus musculus GN=Ahsg PE=1 SV=1	47	6	29.57
2	Carboxylesterase 1D OS=Mus musculus GN=Ces1d PE=1 SV=1	44	6	11.15
2	Apolipoprotein A-II OS=Mus musculus GN=Apoa2 PE=1 SV=2	31	3	14.71
2	Murinoglobulin-2 OS=Mus musculus GN=Mug2 PE=2 SV=2	157	19	11.72
2	Apolipoprotein A-IV OS=Mus musculus GN=Apoa4 PE=1 SV=3	65	13	36.71
2	Liver carboxylesterase 1 OS=Mus musculus GN=Ces1 PE=2 SV=1	35	5	10.8
2	Corticosteroid-binding globulin OS=Mus musculus GN=Serpina6 PE=1 SV=1	23	5	17.38
2	Isoform 2 of Ig mu chain C region OS=Mus musculus GN=Ighm	73	11	25.05
2	Ig mu chain C region OS=Mus musculus GN=Ighm PE=1 SV=2	73	11	26.21
2	Serine protease inhibitor A3N OS=Mus musculus GN=Serpina3n PE=1 SV=1	55	8	17.46
2	Serine protease inhibitor A3C OS=Mus musculus GN=Serpina3c PE=2 SV=1	43	5	6.47
2	Serine protease inhibitor A3G OS=Mus musculus GN=Serpina3g PE=2 SV=2	43	5	6.14
2	Apolipoprotein E OS=Mus musculus GN=Apoe PE=1 SV=2	30	4	16.4
2	Serine protease inhibitor A3F OS=Mus musculus GN=Serpina3f PE=1 SV=3	38	4	5.84
2	Isoform Short of Complement C3 OS=Mus musculus GN=C3	51	7	16.82

(continued on next page)

Table S7. Identified proteins in control group (three technical replicates) (continued)

Replicate number	Protein Description	Protein Matched Products	Protein matched Peptides	Protein seq Cover (%)
2	Isoform 2 of Fibrinogen alpha chain OS=Mus musculus GN=Fga	64	13	27.29
2	Fibrinogen alpha chain OS=Mus musculus GN=Fga PE=2 SV=1	64	13	19.26
2	Beta-2-microglobulin OS=Mus musculus GN=B2m PE=1 SV=2	11	2	7.56
2	Ceruloplasmin OS=Mus musculus GN=Cp PE=1 SV=2	125	21	29.88
2	Complement factor B OS=Mus musculus GN=Cfb PE=1 SV=2	77	13	19.97
2	Ig heavy chain V region AC38 205.12 OS=Mus musculus PE=1 SV=1	9	2	16.1
2	Ig heavy chain V region J558 OS=Mus musculus PE=1 SV=1	9	2	16.24
2	Ig heavy chain V region MOPC 104E OS=Mus musculus PE=1 SV=1	9	2	16.24
2	Histidine-rich glycoprotein OS=Mus musculus GN=Hrg PE=1 SV=2	51	9	16.57
2	Clusterin OS=Mus musculus GN=Clu PE=1 SV=1	52	10	20.98
2	Ig kappa chain V-III region MOPC 70 OS=Mus musculus PE=1 SV=1	17	2	30.63
2	Ig kappa chain V-III region PC 7132 OS=Mus musculus PE=1 SV=1	17	2	30.36
2	Ig kappa chain V-III region 50S10.1 OS=Mus musculus PE=1 SV=1	17	2	30.63
2	Ig kappa chain V-III region PC 2880/PC 1229 OS=Mus musculus PE=1 SV=1	17	2	30.63
2	Serum paraoxonase/arylesterase 1 OS=Mus musculus GN=Pon1 PE=1 SV=2	23	5	21.13
2	Vitamin D-binding protein OS=Mus musculus GN=Gc PE=1 SV=2	70	11	22.9
2	Apolipoprotein C-III OS=Mus musculus GN=Apoc3 PE=1 SV=2	9	2	38.38
2	Ig kappa chain V-III region PC 6684 OS=Mus musculus PE=1 SV=1	11	1	16.22
2	Ig kappa chain V-III region PC 7769 OS=Mus musculus PE=1 SV=1	11	1	16.22
2	Ig kappa chain V-III region PC 7175 OS=Mus musculus PE=1 SV=1	11	1	16.22
2	Ig kappa chain V-III region PC 2485/PC 4039 OS=Mus musculus PE=1 SV=1	11	1	16.22
2	Ig kappa chain V-III region PC 7940 OS=Mus musculus PE=1 SV=1	11	1	16.22
2	Ig kappa chain V-III region PC 7183 OS=Mus musculus PE=1 SV=1	11	1	16.22
2	Ig kappa chain V-III region PC 7210 OS=Mus musculus PE=1 SV=1	11	1	16.36
2	Ig kappa chain V-III region PC 6308 OS=Mus musculus PE=1 SV=1	11	1	16.22
2	Ig kappa chain V-III region PC 7043 OS=Mus musculus PE=1 SV=1	11	1	16.22
2	Ig kappa chain V-III region CBPC 101 OS=Mus musculus PE=1 SV=1	11	1	16.22
2	Ig kappa chain V-III region PC 3741/TEPC 111 OS=Mus musculus PE=1 SV=1	11	1	16.22

(continued on next page)

Table S7. Identified proteins in control group (three technical replicates) (continued)

Replicate number	Protein Description	Protein Matched Products	Protein matched Peptides	Protein seq Cover (%)
2	Ig kappa chain V-III region TEPC 124 OS=Mus musculus PE=1 SV=1	11	1	16.07
2	Ig kappa chain V-III region PC 2413 OS=Mus musculus PE=1 SV=1	11	1	16.22
2	Fibrinogen gamma chain OS=Mus musculus GN=Fgg PE=1 SV=1	46	12	28.44
2	Fibrinogen beta chain OS=Mus musculus GN=Fgb PE=2 SV=1	60	11	23.28
2	Mannose-binding protein C OS=Mus musculus GN=Mbl2 PE=2 SV=2	18	3	15.98
2	Inter-alpha-trypsin inhibitor heavy chain H2 OS=Mus musculus GN=Itih2 PE=1 SV=1	62	12	12.16
2	Isoform 2 of Ig gamma-2B chain C region OS=Mus musculus GN=Igh-3	13	4	12.84
2	Ig gamma-2B chain C region OS=Mus musculus GN=Igh-3 PE=1 SV=3	13	4	10.64
2	Complement component C8 gamma chain OS=Mus musculus GN=C8g PE=1 SV=1	19	3	17.33
2	Fetuin-B OS=Mus musculus GN=Fetub PE=1 SV=1	15	3	9.54
2	Plasminogen OS=Mus musculus GN=Plg PE=1 SV=3	46	10	12.68
2	Antithrombin-III OS=Mus musculus GN=Serpinc1 PE=1 SV=1	48	9	23.23
2	Gelsolin OS=Mus musculus GN=Gsn PE=1 SV=3	64	17	23.33
2	Isoform 2 of Gelsolin OS=Mus musculus GN=Gsn	61	16	23.94
2	Plasma protease C1 inhibitor OS=Mus musculus GN=Serping1 PE=1 SV=3	36	7	24.21
2	Ig kappa chain V-VI region NQ2-6.1 OS=Mus musculus PE=2 SV=1	6	1	14.81
2	Alpha-2-macroglobulin-P OS=Mus musculus GN=A2mp PE=2 SV=2	26	5	4.14
2	Prothrombin OS=Mus musculus GN=F2 PE=1 SV=1	45	12	22.65
2	Alpha-2-antiplasmin OS=Mus musculus GN=Serpinf2 PE=1 SV=1	33	9	21.59
2	Carboxypeptidase N catalytic chain OS=Mus musculus GN=Cpn1 PE=2 SV=1	15	5	15.97
2	Reversed Sequence 25008	10	2	14.63
2	Complement factor H OS=Mus musculus GN=Cfh PE=1 SV=2	51	11	11.35
2	Lumican OS=Mus musculus GN=Lum PE=1 SV=2	8	3	11.24
2	Isoform 2 of Interleukin-1 receptor accessory protein OS=Mus musculus GN=Il1rap	8	2	5.28
2	Interleukin-1 receptor accessory protein OS=Mus musculus GN=Il1rap PE=1 SV=1	8	2	3.33
2	PRKR-interacting protein 1 OS=Mus musculus GN=Prkrip1 PE=1 SV=2	8	2	10.22
2	Carboxypeptidase N subunit 2 OS=Mus musculus GN=Cpn2 PE=1 SV=2	22	5	7.13

(continued on next page)

Table S7. Identified proteins in control group (three technical replicates) (continued)

Replicate number	Protein Description	Protein Matched Products	Protein matched Peptides	Protein seq Cover (%)
2	Heparin cofactor 2 OS=Mus musculus GN=Serpind1 PE=1 SV=1	12	3	6.07
2	Ras association domain-containing protein 1 OS=Mus musculus GN=Rassf1 PE=2 SV=1	15	5	18.24
2	Isoform 3 of Afamin OS=Mus musculus GN=Afm	40	11	23.73
2	Afamin OS=Mus musculus GN=Afm PE=1 SV=2	40	11	23.85
2	Inter alpha-trypsin inhibitor_ heavy chain 4 OS=Mus musculus GN=Itih4 PE=1 SV=2	35	10	12.21
2	Isoform 2 of Inter alpha-trypsin inhibitor_ heavy chain 4 OS=Mus musculus GN=Itih4	31	9	11.18
2	Zinc-alpha-2-glycoprotein OS=Mus musculus GN=Azgp1 PE=1 SV=2	9	4	7.49
2	Isoform 2 of Afamin OS=Mus musculus GN=Afm	34	8	26.05
2	Inter-alpha-trypsin inhibitor heavy chain H1 OS=Mus musculus GN=Itih1 PE=1 SV=2	27	8	12.57
2	Ig gamma-2A chain C region secreted form OS=Mus musculus PE=1 SV=1	8	4	8.36
2	Protein AMBP OS=Mus musculus GN=Ambp PE=2 SV=2	9	2	9.17
2	Reversed Sequence 22519	5	1	12.57
2	Alpha-enolase OS=Mus musculus GN=Eno1 PE=1 SV=3	10	1	1.38
2	Reversed Sequence 9051	7	2	11.93
2	Reversed Sequence 12908	15	2	5.53
3	Serum albumin OS=Mus musculus GN=Alb PE=1 SV=3	791	64	66.45
3	Apolipoprotein A-I OS=Mus musculus GN=Apoa1 PE=1 SV=2	244	21	46.21
3	Serotransferrin OS=Mus musculus GN=Tf PE=1 SV=1	619	52	51.51
3	Serine protease inhibitor A3K OS=Mus musculus GN=Serpina3k PE=1 SV=2	219	17	33.49
3	Hemoglobin subunit alpha OS=Mus musculus GN=Hba PE=1 SV=2	73	6	55.63
3	Alpha-1-antitrypsin 1-4 OS=Mus musculus GN=Serpina1d PE=2 SV=1	247	21	39.71
3	Alpha-2-macroglobulin OS=Mus musculus GN=A2m PE=1 SV=3	704	58	35.59
3	Alpha-1-antitrypsin 1-2 OS=Mus musculus GN=Serpina1b PE=1 SV=2	225	18	37.77
3	Alpha-1-antitrypsin 1-3 OS=Mus musculus GN=Serpina1c PE=1 SV=2	218	19	39.56
3	Alpha-1-antitrypsin 1-1 OS=Mus musculus GN=Serpina1a PE=1 SV=4	218	19	39.47
3	Alpha-1-antitrypsin 1-5 OS=Mus musculus GN=Serpina1e PE=1 SV=1	140	13	33.41
3	Hemoglobin subunit beta-1 OS=Mus musculus GN=Hbb-b1 PE=1 SV=2	53	7	49.66

(continued on next page)

Table S7. Identified proteins in control group (three technical replicates) (continued)

Replicate number	Protein Description	Protein Matched Products	Protein matched Peptides	Protein seq Cover (%)
3	Serine protease inhibitor A3M OS=Mus musculus GN=Serpina3m PE=1 SV=2	89	7	16.03
3	Enolase 1 OS=Saccharomyces cerevisiae (st	125	14	29.29
3	Carboxylesterase 1C OS=Mus musculus GN=Ces1c PE=1 SV=4	164	17	32.31
3	Hemoglobin subunit beta-2 OS=Mus musculus GN=Hbb-b2 PE=1 SV=2	35	3	15.65
3	Alpha-2-HS-glycoprotein OS=Mus musculus GN=Ahsg PE=1 SV=1	48	4	22.61
3	Hemopexin OS=Mus musculus GN=Hpx PE=1 SV=2	156	21	44.78
3	Complement C3 OS=Mus musculus GN=C3 PE=1 SV=3	303	43	28.2
3	Murinoglobulin-1 OS=Mus musculus GN=Mug1 PE=1 SV=3	300	37	27.03
3	Apolipoprotein A-II OS=Mus musculus GN=Apoa2 PE=1 SV=2	36	3	34.31
3	Beta-2-glycoprotein 1 OS=Mus musculus GN=Apoh PE=1 SV=1	80	12	44.35
3	Hemoglobin subunit epsilon-Y2 OS=Mus musculus GN=Hbb-y PE=1 SV=2	18	2	6.8
3	Kininogen-1 OS=Mus musculus GN=Kng1 PE=1 SV=1	107	16	32.83
3	Isoform 3 of Kininogen-1 OS=Mus musculus GN=Kng1	99	15	41.46
3	Isoform LMW of Kininogen-1 OS=Mus musculus GN=Kng1	90	14	43.29
3	Apolipoprotein A-IV OS=Mus musculus GN=Apoa4 PE=1 SV=3	67	13	31.9
3	Murinoglobulin-2 OS=Mus musculus GN=Mug2 PE=2 SV=2	193	21	10.96
3	Corticosteroid-binding globulin OS=Mus musculus GN=Serpina6 PE=1 SV=1	44	6	16.37
3	Clusterin OS=Mus musculus GN=Clu PE=1 SV=1	72	14	41.07
3	Isoform 2 of Ig mu chain C region OS=Mus musculus GN=Ighm	78	11	25.47
3	Ig mu chain C region OS=Mus musculus GN=Ighm PE=1 SV=2	78	11	26.65
3	Ig kappa chain V-III region MOPC 70 OS=Mus musculus PE=1 SV=1	17	2	30.63
3	Ig kappa chain V-III region PC 7132 OS=Mus musculus PE=1 SV=1	17	2	30.36
3	Ig kappa chain V-III region 50S10.1 OS=Mus musculus PE=1 SV=1	17	2	30.63
3	Ig kappa chain V-III region PC 2880/PC 1229 OS=Mus musculus PE=1 SV=1	17	2	30.63
3	Antithrombin-III OS=Mus musculus GN=Serpinc1 PE=1 SV=1	64	11	21.08
3	Carboxylesterase 1D OS=Mus musculus GN=Ces1d PE=1 SV=1	44	4	9.2
3	Serine protease inhibitor A3F OS=Mus musculus GN=Serpina3f PE=1 SV=3	33	4	9.44
3	Ig kappa chain V-III region PC 6684 OS=Mus musculus PE=1 SV=1	11	1	16.22
3	Ig kappa chain V-III region PC 7769 OS=Mus musculus PE=1 SV=1	11	1	16.22

(continued on next page)

Table S7. Identified proteins in control group (three technical replicates) (continued)

Replicate number	Protein Description	Protein Matched Products	Protein matched Peptides	Protein seq Cover (%)
3	Ig kappa chain V-III region PC 7175 OS=Mus musculus PE=1 SV=1	11	1	16.22
3	Ig kappa chain V-III region PC 2485/PC 4039 OS=Mus musculus PE=1 SV=1	11	1	16.22
3	Ig kappa chain V-III region PC 7940 OS=Mus musculus PE=1 SV=1	11	1	16.22
3	Ig kappa chain V-III region PC 7183 OS=Mus musculus PE=1 SV=1	11	1	16.22
3	Ig kappa chain V-III region PC 7210 OS=Mus musculus PE=1 SV=1	11	1	16.36
3	Ig kappa chain V-III region PC 6308 OS=Mus musculus PE=1 SV=1	11	1	16.22
3	Ig kappa chain V-III region PC 7043 OS=Mus musculus PE=1 SV=1	11	1	16.22
3	Ig kappa chain V-III region CBPC 101 OS=Mus musculus PE=1 SV=1	11	1	16.22
3	Ig kappa chain V-III region PC 3741/TEPC 111 OS=Mus musculus PE=1 SV=1	11	1	16.22
3	Ig kappa chain V-III region TEPC 124 OS=Mus musculus PE=1 SV=1	11	1	16.07
3	Ig kappa chain V-III region PC 2413 OS=Mus musculus PE=1 SV=1	11	1	16.22
3	Serine protease inhibitor A3C OS=Mus musculus GN=Serpina3c PE=2 SV=1	30	3	6.24
3	Serine protease inhibitor A3N OS=Mus musculus GN=Serpina3n PE=1 SV=1	30	3	6.22
3	Serine protease inhibitor A3G OS=Mus musculus GN=Serpina3g PE=2 SV=2	30	3	5.91
3	Isoform Short of Complement C3 OS=Mus musculus GN=C3	69	9	21.87
3	Fibrinogen gamma chain OS=Mus musculus GN=Fgg PE=1 SV=1	71	11	28.67
3	Apolipoprotein E OS=Mus musculus GN=Apoe PE=1 SV=2	35	7	30.23
3	Ig heavy chain V region MOPC 104E OS=Mus musculus PE=1 SV=1	15	3	44.44
3	Mannose-binding protein C OS=Mus musculus GN=Mbl2 PE=2 SV=2	24	3	15.98
3	Ig heavy chain V region AC38 205.12 OS=Mus musculus PE=1 SV=1	13	2	27.97
3	Ig heavy chain V region J558 OS=Mus musculus PE=1 SV=1	13	2	28.21
3	Histidine-rich glycoprotein OS=Mus musculus GN=Hrg PE=1 SV=2	56	11	18.48
3	Serum paraoxonase/arylesterase 1 OS=Mus musculus GN=Pon1 PE=1 SV=2	16	2	8.45
3	Liver carboxylesterase 1 OS=Mus musculus GN=Ces1 PE=2 SV=1	27	2	2.83
3	Isoform 2 of Fibrinogen alpha chain OS=Mus musculus GN=Fga	71	13	31.42
3	Fibrinogen alpha chain OS=Mus musculus GN=Fga PE=2 SV=1	74	14	24.08
3	Apolipoprotein C-III OS=Mus musculus GN=Apoc3 PE=1 SV=2	6	1	19.19

(continued on next page)

Table S7. Identified proteins in control group (three technical replicates) (continued)

Replicate number	Protein Description	Protein Matched Products	Protein matched Peptides	Protein seq Cover (%)
3	Vitamin D-binding protein OS=Mus musculus GN=Gc PE=1 SV=2	96	13	25.21
3	Fibrinogen beta chain OS=Mus musculus GN=Fgb PE=2 SV=1	67	13	30.35
3	Alpha-2-antiplasmin OS=Mus musculus GN=Serpinf2 PE=1 SV=1	57	9	21.38
3	Prothrombin OS=Mus musculus GN=F2 PE=1 SV=1	87	15	31.88
3	Ceruloplasmin OS=Mus musculus GN=Cp PE=1 SV=2	92	12	14.89
3	Complement factor B OS=Mus musculus GN=Cfb PE=1 SV=2	89	16	26.02
3	Inter-alpha-trypsin inhibitor heavy chain H2 OS=Mus musculus GN=Itih2 PE=1 SV=1	69	10	13.53
3	Fetuin-B OS=Mus musculus GN=Fetub PE=1 SV=1	32	6	20.1
3	Beta-2-microglobulin OS=Mus musculus GN=B2m PE=1 SV=2	7	1	7.56
3	Protein AMBP OS=Mus musculus GN=Ambp PE=2 SV=2	16	3	13.75
3	Isoform 2 of Ig gamma-2B chain C region OS=Mus musculus GN=Igh-3	15	3	16.72
3	Ig gamma-2B chain C region OS=Mus musculus GN=Igh-3 PE=1 SV=3	15	3	13.86
3	Plasminogen OS=Mus musculus GN=Plg PE=1 SV=3	78	18	22.17
3	Plasma protease C1 inhibitor OS=Mus musculus GN=Serping1 PE=1 SV=3	26	5	13.69
3	Gelsolin OS=Mus musculus GN=Gsn PE=1 SV=3	54	12	15.77
3	Complement factor H OS=Mus musculus GN=Cfh PE=1 SV=2	68	17	23.26
3	Isoform 2 of Gelsolin OS=Mus musculus GN=Gsn	51	11	15.87
3	Ig kappa chain V-VI region NQ2-6.1 OS=Mus musculus PE=2 SV=1	6	1	14.81
3	Major urinary proteins 11 and 8 (Fragment) OS=Mus musculus GN=Mup8 PE=2 SV=1	19	4	27.15
3	Major urinary protein 6 OS=Mus musculus GN=Mup6 PE=1 SV=2	19	4	22.78
3	Major urinary protein 17 OS=Mus musculus GN=Mup17 PE=2 SV=2	16	3	22.78
3	Carboxypeptidase N subunit 2 OS=Mus musculus GN=Cpn2 PE=1 SV=2	28	6	10.6
3	Lumican OS=Mus musculus GN=Lum PE=1 SV=2	22	5	13.61
3	Epidermal growth factor receptor OS=Mus musculus GN=Egfr PE=1 SV=1	31	6	5.12
3	Alpha-2-macroglobulin-P OS=Mus musculus GN=A2mp PE=2 SV=2	24	7	5.97
3	Glutathione peroxidase 3 OS=Mus musculus GN=Gpx3 PE=2 SV=2	6	2	10.18
3	Isoform 3 of Afamin OS=Mus musculus GN=Afm	33	9	22.42
3	Afamin OS=Mus musculus GN=Afm PE=1 SV=2	33	9	22.53

(continued on next page)

Table S7. Identified proteins in control group (three technical replicates) (continued)

Replicate number	Protein Description	Protein Matched Products	Protein matched Peptides	Protein seq Cover (%)
3	Keratin_ type II cytoskeletal 75 OS=Mus musculus GN=Krt75 PE=1 SV=1	10	1	2.18
3	Keratin_ type II cytoskeletal 6A OS=Mus musculus GN=Krt6a PE=1 SV=3	15	2	3.98
3	Keratin_ type II cytoskeletal 6B OS=Mus musculus GN=Krt6b PE=1 SV=3	10	1	2.14
3	Keratin_ type II cytoskeletal 5 OS=Mus musculus GN=Krt5 PE=1 SV=1	24	4	8.28
3	Heparin cofactor 2 OS=Mus musculus GN=Serpind1 PE=1 SV=1	18	6	15.27
3	Carboxypeptidase N catalytic chain OS=Mus musculus GN=Cpn1 PE=2 SV=1	15	5	19.04
3	Major urinary protein 2 OS=Mus musculus GN=Mup2 PE=1 SV=1	12	3	16.11
3	Major urinary protein 1 OS=Mus musculus GN=Mup1 PE=1 SV=1	12	3	16.11
3	Phosphatidylinositol-glycan-specific phospholipase D OS=Mus musculus GN=Gpld1 PE=1 SV=1	30	6	8.6
3	Isoform 2 of Afamin OS=Mus musculus GN=Afm	30	6	20
3	Plasma kallikrein OS=Mus musculus GN=Klkb1 PE=1 SV=2	33	12	20.85
3	Isoform 2 of Inter alpha-trypsin inhibitor_ heavy chain 4 OS=Mus musculus GN=Itih4	51	12	15.95
3	Inter alpha-trypsin inhibitor_ heavy chain 4 OS=Mus musculus GN=Itih4 PE=1 SV=2	51	12	15.29
3	Angiotensinogen OS=Mus musculus GN=Agt PE=1 SV=1	11	2	4.61
3	Isocitrate dehydrogenase [NADP] cytoplasmic OS=Mus musculus GN=Idh1 PE=1 SV=2	12	2	5.56
3	Ig kappa chain V-II region 26-10 OS=Mus musculus PE=1 SV=1	7	1	11.5
3	Complement C5 OS=Mus musculus GN=C5 PE=1 SV=2	52	14	8.63
3	Isoform 2 of Interleukin-1 receptor accessory protein OS=Mus musculus GN=Il1rap	15	5	11.11
3	Interleukin-1 receptor accessory protein OS=Mus musculus GN=Il1rap PE=1 SV=1	15	6	10
3	Complement component C9 OS=Mus musculus GN=C9 PE=1 SV=2	26	9	18.61
3	Complement C1q subcomponent subunit B OS=Mus musculus GN=C1qb PE=1 SV=2	9	3	15.42
3	Carboxylesterase 1E OS=Mus musculus GN=Ces1e PE=1 SV=1	10	1	1.25
3	Proline-rich transmembrane protein 4 OS=Mus musculus GN=Prrt4 PE=2 SV=3	8	2	2.99
3	Isoform 5 of Cation channel sperm-associated protein subunit gamma 1 OS=Mus musculus GN=Catsperg1	5	2	2.23
3	Isoform 3 of Cation channel sperm-associated protein subunit gamma 1 OS=Mus musculus GN=Catsperg1	5	2	1

(continued on next page)

Table S7. Identified proteins in control group (three technical replicates) (continued)

Replicate number	Protein Description	Protein Matched Products	Protein matched Peptides	Protein seq Cover (%)
3	Isoform 2 of Cation channel sperm-associated protein subunit gamma 1 OS=Mus musculus GN=Catsperg1	5	2	0.73
3	Cation channel sperm-associated protein subunit gamma 1 OS=Mus musculus GN=Catsperg1 PE=2 SV=1	5	2	0.7
3	Cation channel sperm-associated protein subunit gamma 2 OS=Mus musculus GN=Catsperg2 PE=1 SV=1	4	1	0.7
3	Isoform 6 of Cation channel sperm-associated protein subunit gamma 1 OS=Mus musculus GN=Catsperg1	4	1	4.17
3	Isoform 4 of Cation channel sperm-associated protein subunit gamma 1 OS=Mus musculus GN=Catsperg1	4	1	3.7
3	Reversed Sequence 15288	2	1	8.97
3	Isoform 3 of DnaJ homolog subfamily A member 3_ mitochondrial OS=Mus musculus GN=Dnaja3	6	2	6.99
3	DnaJ homolog subfamily A member 3_ mitochondrial OS=Mus musculus GN=Dnaja3 PE=1 SV=1	6	2	6.25
3	H-2 class I histocompatibility antigen_ Q10 alpha chain OS=Mus musculus GN=H2-Q10 PE=1 SV=3	12	2	7.69
3	Isoform 5 of Actin-binding LIM protein 1 OS=Mus musculus GN=Ablim1	17	4	19.4
3	Toll/interleukin-1 receptor domain-containing adapter protein OS=Mus musculus GN=Tirap PE=1 SV=1	5	2	7.47
3	Isoform 2 of Protein Z-dependent protease inhibitor OS=Mus musculus GN=Serpina10	10	3	4.31
3	Protein Z-dependent protease inhibitor OS=Mus musculus GN=Serpina10 PE=1 SV=1	10	3	3.79
3	Inter-alpha-trypsin inhibitor heavy chain H1 OS=Mus musculus GN=Itih1 PE=1 SV=2	26	6	7.28
3	Reversed Sequence 12561	10	2	4.39
3	Reversed Sequence 21325	7	2	16.73
3	Reversed Sequence 11292	16	4	12.07
3	Isoform 3 of Uncharacterized protein C1orf112 homolog OS=Mus musculus	12	5	4.14
3	Isoform 2 of Uncharacterized protein C1orf112 homolog OS=Mus musculus	12	5	3.78
3	Uncharacterized protein C1orf112 homolog OS=Mus musculus PE=2 SV=2	12	5	3.88
3	Transcription elongation factor A protein-like 1 OS=Mus musculus GN=Tceal1 PE=2 SV=1	5	1	7.27
3	Reversed Sequence 1868	3	1	8.45

Table S8. Identified proteins in group treated with 200 mg kg^{-1} day^{-1} of diacetyl (three technical replicates)

Replicate number	Protein description	Protein Matched Products	Protein Matched Peptides	Protein seqCover (%)
1	Apolipoprotein A-I OS=Mus musculus GN=Apoa1 PE=1 SV=2	153	14	54.55
1	Serum albumin OS=Mus musculus GN=Alb PE=1 SV=3	477	43	56.58
1	Hemoglobin subunit alpha OS=Mus musculus GN=Hba PE=1 SV=2	83	8	55.63
1	Hemoglobin subunit beta-1 OS=Mus musculus GN=Hbb-b1 PE=1 SV=2	56	7	59.18
1	Alpha-1-antitrypsin 1-4 OS=Mus musculus GN=Serpina1d PE=2 SV=1	181	19	46.49
1	Serotransferrin OS=Mus musculus GN=Tf PE=1 SV=1	379	44	47.06
1	Serine protease inhibitor A3K OS=Mus musculus GN=Serpina3k PE=1 SV=2	141	13	37.56
1	Hemoglobin subunit beta-2 OS=Mus musculus GN=Hbb-b2 PE=1 SV=2	35	5	35.37
1	Alpha-1-antitrypsin 1-5 OS=Mus musculus GN=Serpina1e PE=1 SV=1	111	12	32.45
1	Alpha-1-antitrypsin 1-2 OS=Mus musculus GN=Serpina1b PE=1 SV=2	161	18	44.55
1	Alpha-2-macroglobulin OS=Mus musculus GN=A2m PE=1 SV=3	364	37	29.1
1	Alpha-1-antitrypsin 1-3 OS=Mus musculus GN=Serpina1c PE=1 SV=2	151	19	44.66
1	Alpha-1-antitrypsin 1-1 OS=Mus musculus GN=Serpina1a PE=1 SV=4	151	19	44.55
1	Apolipoprotein A-IV OS=Mus musculus GN=Apoa4 PE=1 SV=3	81	11	36.71
1	Hemoglobin subunit epsilon-Y2 OS=Mus musculus GN=Hbb-y PE=1 SV=2	11	1	6.8
1	Enolase 1 OS=Saccharomyces cerevisiae (st	124	15	41.88
1	Serine protease inhibitor A3M OS=Mus musculus GN=Serpina3m PE=1 SV=2	55	7	15.55
1	Apolipoprotein A-II OS=Mus musculus GN=Apoa2 PE=1 SV=2	28	2	14.71
1	Carboxylesterase 1C OS=Mus musculus GN=Ces1c PE=1 SV=4	79	13	31.59
1	Apolipoprotein E OS=Mus musculus GN=Apoe PE=1 SV=2	30	5	16.08
1	Murinoglobulin-1 OS=Mus musculus GN=Mug1 PE=1 SV=3	158	25	18.83
1	Complement C3 OS=Mus musculus GN=C3 PE=1 SV=3	124	23	19.18
1	Transthyretin OS=Mus musculus GN=Ttr PE=1 SV=1	34	5	46.26

(continued on next page)

Table S8. Identified proteins in group treated with 200 mg kg^{-1} day^{-1} of diacetyl (three technical replicates) (cont.)

Replicate number	Protein description	Protein Matched Products	Protein Matched Peptides	Protein seqCover (%)
1	Isoform 2 of Fibrinogen alpha chain OS=Mus musculus GN=Fga	64	12	26.03
1	Fibrinogen alpha chain OS=Mus musculus GN=Fga PE=2 SV=1	64	12	18.38
1	Serine protease inhibitor A3G OS=Mus musculus GN=Serpina3g PE=2 SV=2	36	7	15.45
1	Serine protease inhibitor A3C OS=Mus musculus GN=Serpina3c PE=2 SV=1	33	4	6.47
1	Serine protease inhibitor A3N OS=Mus musculus GN=Serpina3n PE=1 SV=1	33	4	6.46
1	Serine protease inhibitor A3F OS=Mus musculus GN=Serpina3f PE=1 SV=3	34	7	16.85
1	Alpha-2-HS-glycoprotein OS=Mus musculus GN=Ahsg PE=1 SV=1	24	1	6.09
1	Kininogen-1 OS=Mus musculus GN=Kng1 PE=1 SV=1	67	11	23.45
1	Clusterin OS=Mus musculus GN=Clu PE=1 SV=1	40	6	14.51
1	Isoform 3 of Kininogen-1 OS=Mus musculus GN=Kng1	58	9	25.63
1	Carboxylesterase 1D OS=Mus musculus GN=Ces1d PE=1 SV=1	25	4	9.91
1	Antithrombin-III OS=Mus musculus GN=Serpinc1 PE=1 SV=1	65	17	42.58
1	Murinoglobulin-2 OS=Mus musculus GN=Mug2 PE=2 SV=2	88	13	9.3
1	Isoform LMW of Kininogen-1 OS=Mus musculus GN=Kng1	48	8	25.69
1	Major urinary proteins 11 and 8 (Fragment) OS=Mus musculus GN=Mup8 PE=2 SV=1	7	3	27.81
1	Major urinary protein 1 OS=Mus musculus GN=Mup1 PE=1 SV=1	7	3	23.33
1	Major urinary protein 17 OS=Mus musculus GN=Mup17 PE=2 SV=2	7	3	23.33
1	Major urinary protein 6 OS=Mus musculus GN=Mup6 PE=1 SV=2	7	3	23.33
1	Major urinary protein 2 OS=Mus musculus GN=Mup2 PE=1 SV=1	6	2	14.44
1	Hemopexin OS=Mus musculus GN=Hpx PE=1 SV=2	47	9	31.96
1	Vitamin D-binding protein OS=Mus musculus GN=Gc PE=1 SV=2	59	9	13.45
1	Major urinary protein 5 OS=Mus musculus GN=Mup5 PE=2 SV=1	5	1	6.11
1	Liver carboxylesterase 1 OS=Mus musculus GN=Ces1 PE=2 SV=1	11	1	1.59

(continued on next page)

Table S8. Identified proteins in group treated with 200 mg kg^{-1} day^{-1} of diacetyl (three technical replicates) (cont.)

Replicate number	Protein description	Protein Matched Products	Protein Matched Peptides	Protein seqCover (%)
1	Isoform Short of Complement C3 OS=Mus musculus GN=C3	15	4	9.72
1	Ig heavy chain V region AC38 205.12 OS=Mus musculus PE=1 SV=1	5	1	16.1
1	Ig heavy chain V region J558 OS=Mus musculus PE=1 SV=1	5	1	16.24
1	Ig heavy chain V region MOPC 104E OS=Mus musculus PE=1 SV=1	5	1	16.24
1	Zinc-alpha-2-glycoprotein OS=Mus musculus GN=Azgp1 PE=1 SV=2	7	3	7.82
1	Histidine-rich glycoprotein OS=Mus musculus GN=Hrg PE=1 SV=2	29	5	11.24
1	Alpha-2-antiplasmin OS=Mus musculus GN=Serpinf2 PE=1 SV=1	14	5	14.26
1	Gelsolin OS=Mus musculus GN=Gsn PE=1 SV=3	54	10	16.28
1	Isoform 2 of Gelsolin OS=Mus musculus GN=Gsn	52	9	16.42
1	Serum paraoxonase/arylesterase 1 OS=Mus musculus GN=Pon1 PE=1 SV=2	8	3	12.96
1	Beta-2-glycoprotein 1 OS=Mus musculus GN=Apoh PE=1 SV=1	35	8	35.94
1	Isoform 2 of Ig mu chain C region OS=Mus musculus GN=Ighm	31	7	17.26
1	Ig mu chain C region OS=Mus musculus GN=Ighm PE=1 SV=2	31	7	18.06
1	Ig kappa chain V-III region PC 6684 OS=Mus musculus PE=1 SV=1	4	1	16.22
1	Ig kappa chain V-III region PC 7769 OS=Mus musculus PE=1 SV=1	4	1	16.22
1	Ig kappa chain V-III region PC 7175 OS=Mus musculus PE=1 SV=1	4	1	16.22
1	Ig kappa chain V-III region PC 2485/PC 4039 OS=Mus musculus PE=1 SV=1	4	1	16.22
1	Ig kappa chain V-III region PC 7940 OS=Mus musculus PE=1 SV=1	4	1	16.22
1	Ig kappa chain V-III region PC 7183 OS=Mus musculus PE=1 SV=1	4	1	16.22
1	Ig kappa chain V-III region PC 7210 OS=Mus musculus PE=1 SV=1	4	1	16.36
1	Ig kappa chain V-III region PC 6308 OS=Mus musculus PE=1 SV=1	4	1	16.22
1	Ig kappa chain V-III region PC 7043 OS=Mus musculus PE=1 SV=1	4	1	16.22

(continued on next page)

Table S8. Identified proteins in group treated with 200 mg kg^{-1} day^{-1} of diacetyl (three technical replicates) (cont.)

Replicate number	Protein description	Protein Matched Products	Protein Matched Peptides	Protein seqCover (%)
1	Ig kappa chain V-III region CBPC 101 OS=Mus musculus PE=1 SV=1	4	1	16.22
1	Ig kappa chain V-III region PC 3741/TEPC 111 OS=Mus musculus PE=1 SV=1	4	1	16.22
1	Ig kappa chain V-III region TEPC 124 OS=Mus musculus PE=1 SV=1	4	1	16.07
1	Ig kappa chain V-III region MOPC 70 OS=Mus musculus PE=1 SV=1	4	1	16.22
1	Ig kappa chain V-III region PC 7132 OS=Mus musculus PE=1 SV=1	4	1	16.07
1	Ig kappa chain V-III region PC 2413 OS=Mus musculus PE=1 SV=1	4	1	16.22
1	Ig kappa chain V-III region 50S10.1 OS=Mus musculus PE=1 SV=1	4	1	16.22
1	Ig kappa chain V-III region PC 2880/PC 1229 OS=Mus musculus PE=1 SV=1	4	1	16.22
1	Fibrinogen beta chain OS=Mus musculus GN=Fgb PE=2 SV=1	43	10	24.53
1	Fetuin-B OS=Mus musculus GN=Fetub PE=1 SV=1	13	5	15.98
1	Plasminogen OS=Mus musculus GN=Plg PE=1 SV=3	52	11	15.15
1	Prothrombin OS=Mus musculus GN=F2 PE=1 SV=1	36	11	19.09
1	Complement factor B OS=Mus musculus GN=Cfb PE=1 SV=2	36	10	14.32
1	Ceruloplasmin OS=Mus musculus GN=Cp PE=1 SV=2	58	15	18.38
1	Complement component C9 OS=Mus musculus GN=C9 PE=1 SV=2	14	5	9.49
1	Isoform 2 of Complement factor D OS=Mus musculus GN=Cfd	12	2	13.95
1	Complement factor D OS=Mus musculus GN=Cfd PE=1 SV=1	12	2	13.9
1	Reversed Sequence 5196	11	2	20.12
1	Inter-alpha-trypsin inhibitor heavy chain H2 OS=Mus musculus GN=Itih2 PE=1 SV=1	37	10	11.21
1	Corticosteroid-binding globulin OS=Mus musculus GN=Serpina6 PE=1 SV=1	12	6	12.59
1	Lumican OS=Mus musculus GN=Lum PE=1 SV=2	11	6	17.46
1	Reversed Sequence 9591	46	15	31.82
1	Plasma protease C1 inhibitor OS=Mus musculus GN=Serping1 PE=1 SV=3	19	3	10.32

(continued on next page)

Table S8. Identified proteins in group treated with 200 mg kg^{-1} day^{-1} of diacetyl (three technical replicates) (cont.)

Replicate number	Protein description	Protein Matched Products	Protein Matched Peptides	Protein seqCover (%)
1	Carboxypeptidase N catalytic chain OS=Mus musculus GN=Cpn1 PE=2 SV=1	12	6	18.82
1	Isoform D of Tumor necrosis factor receptor superfamily member 18 OS=Mus musculus GN=Tnfrsf18	5	1	12.12
1	Isoform C of Tumor necrosis factor receptor superfamily member 18 OS=Mus musculus GN=Tnfrsf18	5	1	7.21
1	Isoform B of Tumor necrosis factor receptor superfamily member 18 OS=Mus musculus GN=Tnfrsf18	5	1	5.44
1	Tumor necrosis factor receptor superfamily member 18 OS=Mus musculus GN=Tnfrsf18 PE=1 SV=1	5	1	7.02
1	Isoform 2 of Ig gamma-2B chain C region OS=Mus musculus GN=Igh-3	8	3	4.78
1	Ig gamma-2B chain C region OS=Mus musculus GN=Igh-3 PE=1 SV=3	8	3	3.96
1	Reversed Sequence 147	8	2	14.29
1	Myelin protein zero-like protein 1 OS=Mus musculus GN=Mpzl1 PE=1 SV=1	7	4	25.93
1	U3 small nucleolar RNA-associated protein 14 homolog A OS=Mus musculus GN=Utp14a PE=1 SV=1	17	5	13.17
1	AP-3 complex subunit mu-1 OS=Mus musculus GN=Ap3m1 PE=1 SV=1	4	1	3.59
1	H-2 class I histocompatibility antigen_ Q10 alpha chain OS=Mus musculus GN=H2-Q10 PE=1 SV=3	6	1	4.62
1	Heparin cofactor 2 OS=Mus musculus GN=Serpind1 PE=1 SV=1	12	5	7.95
1	Isoform 2 of Myelin protein zero-like protein 1 OS=Mus musculus GN=Mpzl1	4	2	23.67
1	Isoform Gamma of Prostaglandin E2 receptor EP3 subtype OS=Mus musculus GN=Ptger3	6	2	10.71
1	Isoform Beta of Prostaglandin E2 receptor EP3 subtype OS=Mus musculus GN=Ptger3	6	2	10.8
1	Prostaglandin E2 receptor EP3 subtype OS=Mus musculus GN=Ptger3 PE=1 SV=1	6	2	10.68
1	Alpha-2-macroglobulin-P OS=Mus musculus GN=A2mp PE=2 SV=2	19	8	8.62
1	Inter alpha-trypsin inhibitor_ heavy chain 4 OS=Mus musculus GN=Itih4 PE=1 SV=2	25	10	7.86
1	Isoform 2 of Inter alpha-trypsin inhibitor_ heavy chain 4 OS=Mus musculus GN=Itih4	25	9	8.19
1	Proline-rich transmembrane protein 4 OS=Mus musculus GN=Prrt4 PE=2 SV=3	5	2	2.33
1	Reversed Sequence 10110	8	3	8.58

(continued on next page)

Table S8. Identified proteins in group treated with 200 mg kg^{-1} day^{-1} of diacetyl (three technical replicates) (cont.)

Replicate number	Protein description	Protein Matched Products	Protein Matched Peptides	Protein seqCover (%)
1	Isoform 3 of Protein CASC4 OS=Mus musculus GN=Casc4	14	4	8.38
1	Isoform 2 of Protein CASC4 OS=Mus musculus GN=Casc4	14	4	7.21
1	Protein CASC4 OS=Mus musculus GN=Casc4 PE=2 SV=1	14	4	6.67
1	Biotin--protein ligase OS=Mus musculus GN=Hlcs PE=2 SV=1	6	3	5.54
1	Isoform 4 of Coiled-coil domain-containing protein 33 OS=Mus musculus GN=Ccdc33	6	3	5.75
1	Isoform 3 of Coiled-coil domain-containing protein 33 OS=Mus musculus GN=Ccdc33	6	3	5.75
1	Coiled-coil domain-containing protein 33 OS=Mus musculus GN=Ccdc33 PE=2 SV=2	6	3	4.26
1	Isoform 4 of Interleukin-1 receptor-associated kinase-like 2 OS=Mus musculus GN=Irak2	11	5	10.23
1	Isoform 2 of Interleukin-1 receptor-associated kinase-like 2 OS=Mus musculus GN=Irak2	11	5	8.54
1	Interleukin-1 receptor-associated kinase-like 2 OS=Mus musculus GN=Irak2 PE=2 SV=2	11	5	7.88
1	FERM_ RhoGEF and pleckstrin domain-containing protein 2 OS=Mus musculus GN=Farp2 PE=1 SV=2	16	9	6.76
1	Fibronectin OS=Mus musculus GN=Fn1 PE=1 SV=4	47	19	9.04
1	tRNA-dihydrouridine(16/17) synthase [NAD(P)(+)]-like OS=Mus musculus GN=Dus1l PE=2 SV=1	6	2	7.79
1	Angiotensinogen OS=Mus musculus GN=Agt PE=1 SV=1	11	3	4.61
1	Zinc finger protein 474 OS=Mus musculus GN=Znf474 PE=2 SV=2	3	2	6.34
1	Complement factor H OS=Mus musculus GN=Cfh PE=1 SV=2	20	8	10.05
1	Isoform 5 of Coiled-coil domain-containing protein 33 OS=Mus musculus GN=Ccdc33	1	1	19.08
1	Short-chain specific acyl-CoA dehydrogenase_ mitochondrial OS=Mus musculus GN=Acads PE=1 SV=2	7	2	10.92
1	Spermatogenesis-associated protein 24 OS=Mus musculus GN=Spata24 PE=1 SV=1	6	2	11.71
1	Proteasome subunit beta type-8 OS=Mus musculus GN=Psmb8 PE=1 SV=2	16	6	28.62
1	Opticin OS=Mus musculus GN=Optc PE=2 SV=2	3	1	14.94
1	Fibrinogen gamma chain OS=Mus musculus GN=Fgg PE=1 SV=1	23	6	23.17
1	Isoform 2 of Serine racemase OS=Mus musculus GN=Srr	9	3	14.65
1	Serine racemase OS=Mus musculus GN=Srr PE=1 SV=1	9	3	13.57

(continued on next page)

Table S8. Identified proteins in group treated with 200 mg kg^{-1} day^{-1} of diacetyl (three technical replicates) (cont.)

Replicate number	Protein description	Protein Matched Products	Protein Matched Peptides	Protein seqCover (%)
1	Protein unc-119 homolog A OS=Mus musculus GN=Unc119 PE=1 SV=1	6	1	7.5
1	FERM_ RhoGEF and pleckstrin domain-containing protein 1 OS=Mus musculus GN=Farp1 PE=1 SV=1	15	8	2.96
1	Deoxyribonuclease-2-beta OS=Mus musculus GN=Dnase2b PE=2 SV=1	9	3	12.99
1	Isoform 2 of E3 ubiquitin-protein ligase SH3RF1 OS=Mus musculus GN=Sh3rf1	17	6	8.93
1	E3 ubiquitin-protein ligase SH3RF1 OS=Mus musculus GN=Sh3rf1 PE=1 SV=2	17	6	8.63
1	DnaJ homolog subfamily C member 14 OS=Mus musculus GN=Dnajc14 PE=2 SV=2	12	3	2.84
1	Nascent polypeptide-associated complex subunit alpha_ muscle-specific form OS=Mus musculus GN=Naca PE=1 SV=2	13	7	2.74
1	E3 ubiquitin-protein ligase SHPRH OS=Mus musculus GN=Shprh PE=1 SV=1	5	1	0.24
1	Reversed Sequence 48	10	3	14.12
1	Isoform 4 of E3 ubiquitin-protein ligase SH3RF1 OS=Mus musculus GN=Sh3rf1	14	5	14.96
1	Isoform 3 of E3 ubiquitin-protein ligase SH3RF1 OS=Mus musculus GN=Sh3rf1	14	5	6.88
1	Reversed Sequence 24017	15	4	7.8
1	Protein virilizer homolog OS=Mus musculus GN=Kiaa1429 PE=1 SV=1	8	3	0.39
1	Carboxyl-terminal PDZ ligand of neuronal nitric oxide synthase protein OS=Mus musculus GN=Nos1ap PE=1 SV=3	11	6	11.93
1	Nascent polypeptide-associated complex subunit alpha OS=Mus musculus GN=Naca PE=1 SV=1	2	1	6.98
2	Apolipoprotein A-I OS=Mus musculus GN=Apoa1 PE=1 SV=2	178	18	30
2	Hemoglobin subunit alpha OS=Mus musculus GN=Hba PE=1 SV=2	92	9	59.09
2	Serum albumin OS=Mus musculus GN=Alb PE=1 SV=3	421	40	80.28
2	Serine protease inhibitor A3K OS=Mus musculus GN=Serpina3k PE=1 SV=2	161	13	58.55
2	Hemoglobin subunit beta-1 OS=Mus musculus GN=Hbb-b1 PE=1 SV=2	59	7	30.86
2	Hemoglobin subunit beta-2 OS=Mus musculus GN=Hbb-b2 PE=1 SV=2	37	4	53.74
2	Serotransferrin OS=Mus musculus GN=Tf PE=1 SV=1	329	38	29.93

(continued on next page)

Table S8. Identified proteins in group treated with 200 mg kg^{-1} day^{-1} of diacetyl (three technical replicates) (cont.)

Replicate number	Protein description	Protein Matched Products	Protein Matched Peptides	Protein seqCover (%)
2	Alpha-1-antitrypsin 1-5 OS=Mus musculus GN=Serpina1e PE=1 SV=1	100	11	42.9
2	Alpha-2-macroglobulin OS=Mus musculus GN=A2m PE=1 SV=3	359	37	21.31
2	Hemoglobin subunit epsilon-Y2 OS=Mus musculus GN=Hbb-y PE=1 SV=2	14	1	31.24
2	Alpha-1-antitrypsin 1-2 OS=Mus musculus GN=Serpina1b PE=1 SV=2	154	14	6.8
2	Alpha-1-antitrypsin 1-4 OS=Mus musculus GN=Serpina1d PE=2 SV=1	154	15	32.93
2	Enolase 1 OS=Saccharomyces cerevisiae (st	150	18	34.38
2	Serine protease inhibitor A3M OS=Mus musculus GN=Serpina3m PE=1 SV=2	76	7	47.14
2	Alpha-1-antitrypsin 1-3 OS=Mus musculus GN=Serpina1c PE=1 SV=2	143	13	11.96
2	Alpha-1-antitrypsin 1-1 OS=Mus musculus GN=Serpina1a PE=1 SV=4	143	13	25.97
2	Apolipoprotein A-IV OS=Mus musculus GN=Apoa4 PE=1 SV=3	90	14	25.91
2	Carboxylesterase 1C OS=Mus musculus GN=Ces1c PE=1 SV=4	105	18	46.08
2	Apolipoprotein A-II OS=Mus musculus GN=Apoa2 PE=1 SV=2	17	1	40.43
2	Murinoglobulin-1 OS=Mus musculus GN=Mug1 PE=1 SV=3	131	17	9.8
2	Serine protease inhibitor A3G OS=Mus musculus GN=Serpina3g PE=2 SV=2	41	6	12.94
2	Serine protease inhibitor A3C OS=Mus musculus GN=Serpina3c PE=2 SV=1	35	4	12.05
2	Serine protease inhibitor A3N OS=Mus musculus GN=Serpina3n PE=1 SV=1	35	4	6.47
2	Kininogen-1 OS=Mus musculus GN=Kng1 PE=1 SV=1	82	14	6.46
2	Serine protease inhibitor A3F OS=Mus musculus GN=Serpina3f PE=1 SV=3	35	6	31.01
2	Complement C3 OS=Mus musculus GN=C3 PE=1 SV=3	152	24	14.83
2	Isoform 3 of Kininogen-1 OS=Mus musculus GN=Kng1	66	10	16.36
2	Isoform LMW of Kininogen-1 OS=Mus musculus GN=Kng1	59	9	30.63
2	Apolipoprotein E OS=Mus musculus GN=Apoe PE=1 SV=2	39	8	31.25
2	Transthyretin OS=Mus musculus GN=Ttr PE=1 SV=1	36	5	28.94
2	Isoform 2 of Fibrinogen alpha chain OS=Mus musculus GN=Fga	64	17	46.26

(continued on next page)

Table S8. Identified proteins in group treated with 200 mg kg^{-1} day^{-1} of diacetyl (three technical replicates) (cont.)

Replicate number	Protein description	Protein Matched Products	Protein Matched Peptides	Protein seqCover (%)
2	Fibrinogen alpha chain OS=Mus musculus GN=Fga PE=2 SV=1	64	17	36.62
2	Hemopexin OS=Mus musculus GN=Hpx PE=1 SV=2	64	13	25.86
2	Carboxylesterase 1D OS=Mus musculus GN=Ces1d PE=1 SV=1	23	3	27.61
2	Alpha-2-HS-glycoprotein OS=Mus musculus GN=Ahsg PE=1 SV=1	21	2	5.31
2	Liver carboxylesterase 1 OS=Mus musculus GN=Ces1 PE=2 SV=1	16	2	11.01
2	Clusterin OS=Mus musculus GN=Clu PE=1 SV=1	40	9	2.83
2	Vitamin D-binding protein OS=Mus musculus GN=Gc PE=1 SV=2	60	9	36.16
2	Ceruloplasmin OS=Mus musculus GN=Cp PE=1 SV=2	74	16	17.44
2	Cytochrome b-c1 complex subunit 9 OS=Mus musculus GN=Uqcr10 PE=1 SV=1	5	1	24.41
2	Complement factor B OS=Mus musculus GN=Cfb PE=1 SV=2	54	12	26.56
2	Murinoglobulin-2 OS=Mus musculus GN=Mug2 PE=2 SV=2	76	12	19.32
2	Antithrombin-III OS=Mus musculus GN=Serpinc1 PE=1 SV=1	46	11	8.34
2	Isoform 2 of Ig gamma-2B chain C region OS=Mus musculus GN=Igh-3	15	2	30.32
2	Ig gamma-2B chain C region OS=Mus musculus GN=Igh-3 PE=1 SV=3	15	2	7.16
2	Plasma protease C1 inhibitor OS=Mus musculus GN=Serping1 PE=1 SV=3	20	4	5.94
2	Ig heavy chain V region AC38 205.12 OS=Mus musculus PE=1 SV=1	5	1	12.3
2	Ig heavy chain V region J558 OS=Mus musculus PE=1 SV=1	5	1	16.1
2	Ig heavy chain V region MOPC 104E OS=Mus musculus PE=1 SV=1	5	1	16.24
2	Alpha-2-antiplasmin OS=Mus musculus GN=Serpinf2 PE=1 SV=1	22	6	16.24
2	Isoform 2 of Complement factor D OS=Mus musculus GN=Cfd	5	1	13.44
2	Complement factor D OS=Mus musculus GN=Cfd PE=1 SV=1	5	1	8.14
2	Beta-2-glycoprotein 1 OS=Mus musculus GN=Apoh PE=1 SV=1	25	6	8.11
2	Fetuin-B OS=Mus musculus GN=Fetub PE=1 SV=1	20	4	24.64

(continued on next page)

Table S8. Identified proteins in group treated with 200 mg kg^{-1} day^{-1} of diacetyl (three technical replicates) (cont.)

Replicate number	Protein description	Protein Matched Products	Protein Matched Peptides	Protein seqCover (%)
2	Coiled-coil domain-containing protein 160 OS=Mus musculus GN=Ccdc160 PE=2 SV=1	22	5	19.85
2	Isoform Short of Complement C3 OS=Mus musculus GN=C3	16	4	23.53
2	Gelsolin OS=Mus musculus GN=Gsn PE=1 SV=3	53	11	9.35
2	Isoform 2 of Gelsolin OS=Mus musculus GN=Gsn	51	10	18.46
2	Fibrinogen beta chain OS=Mus musculus GN=Fgb PE=2 SV=1	53	13	18.74
2	Inter-alpha-trypsin inhibitor heavy chain H2 OS=Mus musculus GN=Itih2 PE=1 SV=1	43	9	28.27
2	Prothrombin OS=Mus musculus GN=F2 PE=1 SV=1	38	8	13.64
2	Plasminogen OS=Mus musculus GN=Plg PE=1 SV=3	45	12	13.59
2	Histidine-rich glycoprotein OS=Mus musculus GN=Hrg PE=1 SV=2	28	7	11.08
2	Reversed Sequence 25102	15	2	12.38
2	Reversed Sequence 15087	4	2	8.83
2	Isoform Gamma of Prostaglandin E2 receptor EP3 subtype OS=Mus musculus GN=Ptger3	6	2	35.71
2	Isoform Beta of Prostaglandin E2 receptor EP3 subtype OS=Mus musculus GN=Ptger3	6	2	3.85
2	Prostaglandin E2 receptor EP3 subtype OS=Mus musculus GN=Ptger3 PE=1 SV=1	6	2	3.88
2	Isoform 2 of Ig mu chain C region OS=Mus musculus GN=Ighm	32	7	3.84
2	Ig mu chain C region OS=Mus musculus GN=Ighm PE=1 SV=2	32	7	15.37
2	Zinc-alpha-2-glycoprotein OS=Mus musculus GN=Azgp1 PE=1 SV=2	11	3	16.08
2	Apolipoprotein C-III OS=Mus musculus GN=Apoc3 PE=1 SV=2	4	1	13.68
2	THO complex subunit 7 homolog OS=Mus musculus GN=Thoc7 PE=1 SV=2	11	2	19.19
2	Ankyrin repeat domain-containing protein 49 OS=Mus musculus GN=Ankrd49 PE=2 SV=1	6	1	11.27
2	Claudin-5 OS=Mus musculus GN=Cldn5 PE=1 SV=2	5	1	8.4
2	Alpha-2-macroglobulin-P OS=Mus musculus GN=A2mp PE=2 SV=2	18	7	7.8
2	Ig kappa chain V-III region PC 6684 OS=Mus musculus PE=1 SV=1	4	1	7.6

(continued on next page)

Table S8. Identified proteins in group treated with 200 mg kg^{-1} day^{-1} of diacetyl (three technical replicates) (cont.)

Replicate number	Protein description	Protein Matched Products	Protein Matched Peptides	Protein seqCover (%)
2	Ig kappa chain V-III region PC 7769 OS=Mus musculus PE=1 SV=1	4	1	16.22
2	Ig kappa chain V-III region PC 7175 OS=Mus musculus PE=1 SV=1	4	1	16.22
2	Ig kappa chain V-III region PC 2485/PC 4039 OS=Mus musculus PE=1 SV=1	4	1	16.22
2	Ig kappa chain V-III region PC 7940 OS=Mus musculus PE=1 SV=1	4	1	16.22
2	Ig kappa chain V-III region PC 7183 OS=Mus musculus PE=1 SV=1	4	1	16.22
2	Ig kappa chain V-III region PC 7210 OS=Mus musculus PE=1 SV=1	4	1	16.22
2	Ig kappa chain V-III region PC 6308 OS=Mus musculus PE=1 SV=1	4	1	16.36
2	Ig kappa chain V-III region PC 7043 OS=Mus musculus PE=1 SV=1	4	1	16.22
2	Ig kappa chain V-III region CBPC 101 OS=Mus musculus PE=1 SV=1	4	1	16.22
2	Ig kappa chain V-III region PC 3741/TEPC 111 OS=Mus musculus PE=1 SV=1	4	1	16.22
2	Ig kappa chain V-III region TEPC 124 OS=Mus musculus PE=1 SV=1	4	1	16.22
2	Ig kappa chain V-III region MOPC 70 OS=Mus musculus PE=1 SV=1	4	1	16.07
2	Ig kappa chain V-III region PC 7132 OS=Mus musculus PE=1 SV=1	4	1	16.22
2	Ig kappa chain V-III region PC 2413 OS=Mus musculus PE=1 SV=1	4	1	16.07
2	Ig kappa chain V-III region 50S10.1 OS=Mus musculus PE=1 SV=1	4	1	16.22
2	Ig kappa chain V-III region PC 2880/PC 1229 OS=Mus musculus PE=1 SV=1	4	1	16.22
2	RNA-directed RNA polymerase L OS=Lymphocytic choriomeningitis virus (strain Armstrong) GN=L PE=1 SV=1	28	11	16.22
2	Inter-alpha-trypsin inhibitor heavy chain H1 OS=Mus musculus GN=Itih1 PE=1 SV=2	29	10	2.67
2	Guanine nucleotide exchange factor VAV2 OS=Mus musculus GN=Vav2 PE=1 SV=1	14	4	15.1
2	Isoform 2 of THO complex subunit 7 homolog OS=Mus musculus GN=Thoc7	7	1	2.88
2	Complement factor H OS=Mus musculus GN=Cfh PE=1 SV=2	31	9	5.84

(continued on next page)

Table S8. Identified proteins in group treated with 200 mg kg^{-1} day^{-1} of diacetyl (three technical replicates) (cont.)

Replicate number	Protein description	Protein Matched Products	Protein Matched Peptides	Protein seqCover (%)
2	Isoform STEP38 of Tyrosine-protein phosphatase non-receptor type 5 OS=Mus musculus GN=Ptpn5	12	4	8.51
2	Tyrosine-protein phosphatase non-receptor type 5 OS=Mus musculus GN=Ptpn5 PE=2 SV=2	12	4	15.03
2	Reversed Sequence 8126	19	4	9.61
2	Alpha-(1_3)-fucosyltransferase 10 OS=Mus musculus GN=Fut10 PE=2 SV=1	9	4	13.16
2	Isoform 2 of Alpha-(1_3)-fucosyltransferase 10 OS=Mus musculus GN=Fut10	7	2	13.93
2	Isoform 3 of Monocarboxylate transporter 5 OS=Mus musculus GN=Slc16a4	4	1	11.93
2	Isoform 2 of Monocarboxylate transporter 5 OS=Mus musculus GN=Slc16a4	4	1	14.17
2	Monocarboxylate transporter 5 OS=Mus musculus GN=Slc16a4 PE=2 SV=1	4	1	10.9
2	Isoform 2 of Inter alpha-trypsin inhibitor_ heavy chain 4 OS=Mus musculus GN=Itih4	30	10	10.2
2	Inter alpha-trypsin inhibitor_ heavy chain 4 OS=Mus musculus GN=Itih4 PE=1 SV=2	30	10	10.74
2	Serum paraoxonase/arylesterase 1 OS=Mus musculus GN=Pon1 PE=1 SV=2	5	3	10.3
2	Coagulation factor X OS=Mus musculus GN=F10 PE=1 SV=1	10	4	6.48
2	Noelin OS=Mus musculus GN=Olfm1 PE=1 SV=1	18	7	3.53
2	Corticosteroid-binding globulin OS=Mus musculus GN=Serpina6 PE=1 SV=1	10	5	6.6
2	Reversed Sequence 1667	21	5	11.59
3	Apolipoprotein A-I OS=Mus musculus GN=Apoa1 PE=1 SV=2	145	15	52.65
3	Serum albumin OS=Mus musculus GN=Alb PE=1 SV=3	419	40	58.55
3	Serine protease inhibitor A3K OS=Mus musculus GN=Serpina3k PE=1 SV=2	149	12	33.97
3	Hemoglobin subunit beta-1 OS=Mus musculus GN=Hbb-b1 PE=1 SV=2	46	6	53.74
3	Hemoglobin subunit alpha OS=Mus musculus GN=Hba PE=1 SV=2	110	11	81.69
3	Hemoglobin subunit beta-2 OS=Mus musculus GN=Hbb-b2 PE=1 SV=2	29	4	29.93
3	Alpha-2-macroglobulin OS=Mus musculus GN=A2m PE=1 SV=3	379	40	31.37
3	Serotransferrin OS=Mus musculus GN=Tf PE=1 SV=1	341	40	37.59

(continued on next page)

Table S8. Identified proteins in group treated with 200 mg kg^{-1} day^{-1} of diacetyl (three technical replicates) (cont.)

Replicate number	Protein description	Protein Matched Products	Protein Matched Peptides	Protein seqCover (%)
3	Hemoglobin subunit epsilon-Y2 OS=Mus musculus GN=Hbb-y PE=1 SV=2	14	1	6.8
3	Alpha-1-antitrypsin 1-5 OS=Mus musculus GN=Serpina1e PE=1 SV=1	95	10	21.07
3	Alpha-1-antitrypsin 1-4 OS=Mus musculus GN=Serpina1d PE=2 SV=1	143	15	30.99
3	Enolase 1 OS=Saccharomyces cerevisiae (st	118	16	39.82
3	Serine protease inhibitor A3M OS=Mus musculus GN=Serpina3m PE=1 SV=2	69	6	11.96
3	Alpha-1-antitrypsin 1-2 OS=Mus musculus GN=Serpina1b PE=1 SV=2	124	13	28.81
3	Apolipoprotein A-IV OS=Mus musculus GN=Apoa4 PE=1 SV=3	85	13	39.24
3	Alpha-1-antitrypsin 1-3 OS=Mus musculus GN=Serpina1c PE=1 SV=2	128	13	25.97
3	Alpha-1-antitrypsin 1-1 OS=Mus musculus GN=Serpina1a PE=1 SV=4	128	13	25.91
3	Carboxylesterase 1C OS=Mus musculus GN=Ces1c PE=1 SV=4	103	16	37.91
3	Apolipoprotein A-II OS=Mus musculus GN=Apoa2 PE=1 SV=2	30	1	9.8
3	Serine protease inhibitor A3G OS=Mus musculus GN=Serpina3g PE=2 SV=2	36	6	15.45
3	Serine protease inhibitor A3C OS=Mus musculus GN=Serpina3c PE=2 SV=1	28	3	6.47
3	Serine protease inhibitor A3N OS=Mus musculus GN=Serpina3n PE=1 SV=1	28	3	6.46
3	Serine protease inhibitor A3F OS=Mus musculus GN=Serpina3f PE=1 SV=3	33	5	15.73
3	Murinoglobulin-1 OS=Mus musculus GN=Mug1 PE=1 SV=3	134	22	19.51
3	Apolipoprotein E OS=Mus musculus GN=Apoe PE=1 SV=2	25	4	16.4
3	Kininogen-1 OS=Mus musculus GN=Kng1 PE=1 SV=1	55	9	20.12
3	Isoform 3 of Kininogen-1 OS=Mus musculus GN=Kng1	46	7	21.04
3	Isoform LMW of Kininogen-1 OS=Mus musculus GN=Kng1	46	7	23.38
3	Complement C3 OS=Mus musculus GN=C3 PE=1 SV=3	135	26	20.69
3	Antithrombin-III OS=Mus musculus GN=Serpinc1 PE=1 SV=1	60	14	31.83
3	Clusterin OS=Mus musculus GN=Clu PE=1 SV=1	31	6	20.98
3	Isoform 2 of Fibrinogen alpha chain OS=Mus musculus GN=Fga	65	10	26.93

(continued on next page)

Table S8. Identified proteins in group treated with 200 mg kg^{-1} day^{-1} of diacetyl (three technical replicates) (cont.)

Replicate number	Protein description	Protein Matched Products	Protein Matched Peptides	Protein seqCover (%)
3	Fibrinogen alpha chain OS=Mus musculus GN=Fga PE=2 SV=1	65	10	19.01
3	Hemopexin OS=Mus musculus GN=Hpx PE=1 SV=2	40	9	21.96
3	Reversed Sequence 17913	8	2	44.44
3	Vitamin D-binding protein OS=Mus musculus GN=Gc PE=1 SV=2	59	10	23.95
3	Transthyretin OS=Mus musculus GN=Ttr PE=1 SV=1	29	5	46.26
3	Zinc-alpha-2-glycoprotein OS=Mus musculus GN=Azgp1 PE=1 SV=2	5	2	8.79
3	Ceruloplasmin OS=Mus musculus GN=Cp PE=1 SV=2	62	16	21.02
3	Carboxylesterase 1D OS=Mus musculus GN=Ces1d PE=1 SV=1	20	5	10.62
3	Apolipoprotein C-III OS=Mus musculus GN=Apoc3 PE=1 SV=2	7	2	38.38
3	Murinoglobulin-2 OS=Mus musculus GN=Mug2 PE=2 SV=2	77	12	8.27
3	Gelsolin OS=Mus musculus GN=Gsn PE=1 SV=3	45	9	17.05
3	Isoform 2 of Gelsolin OS=Mus musculus GN=Gsn	41	7	15.73
3	Beta-2-glycoprotein 1 OS=Mus musculus GN=Apoh PE=1 SV=1	30	7	22.32
3	Isoform Short of Complement C3 OS=Mus musculus GN=C3	18	5	16.64
3	Fetuin-B OS=Mus musculus GN=Fetub PE=1 SV=1	21	4	19.33
3	Inter-alpha-trypsin inhibitor heavy chain H2 OS=Mus musculus GN=Itih2 PE=1 SV=1	57	9	11.42
3	Serum paraoxonase/arylesterase 1 OS=Mus musculus GN=Pon1 PE=1 SV=2	8	2	8.45
3	Complement factor B OS=Mus musculus GN=Cfb PE=1 SV=2	38	10	14.06
3	Isoform 2 of Ig gamma-2B chain C region OS=Mus musculus GN=Igh-3	14	2	7.16
3	Ig gamma-2B chain C region OS=Mus musculus GN=Igh-3 PE=1 SV=3	14	2	5.94
3	Liver carboxylesterase 1 OS=Mus musculus GN=Ces1 PE=2 SV=1	10	2	2.48
3	Alpha-2-antiplasmin OS=Mus musculus GN=Serpinf2 PE=1 SV=1	24	7	16.29
3	Immunoglobulin J chain OS=Mus musculus GN=Igj PE=2 SV=4	7	5	22.64
3	Prothrombin OS=Mus musculus GN=F2 PE=1 SV=1	23	6	8.09

(continued on next page)

Table S8. Identified proteins in group treated with 200 mg kg^{-1} day^{-1} of diacetyl (three technical replicates) (cont.)

Replicate number	Protein description	Protein Matched Products	Protein Matched Peptides	Protein seqCover (%)
3	Histidine-rich glycoprotein OS=Mus musculus GN=Hrg PE=1 SV=2	27	7	15.81
3	Reversed Sequence 21346	3	1	18.18
3	Alpha-2-macroglobulin-P OS=Mus musculus GN=A2mp PE=2 SV=2	35	9	7.33
3	Fibrinogen beta chain OS=Mus musculus GN=Fgb PE=2 SV=1	26	7	15.38
3	Isoform 2 of Ig mu chain C region OS=Mus musculus GN=Ighm	16	5	14.11
3	Ig mu chain C region OS=Mus musculus GN=Ighm PE=1 SV=2	16	5	14.76
3	Carboxypeptidase N catalytic chain OS=Mus musculus GN=Cpn1 PE=2 SV=1	15	5	13.57
3	Major urinary protein 20 OS=Mus musculus GN=Mup20 PE=1 SV=1	9	3	12.15

Table S9. Identified proteins in group treated with 300 mg kg^{-1} day^{-1} of diacetyl (three technical replicates)

Replicate number	Protein Description	Protein Matched Products	Protein matched Peptides	Protein seqCover (%)
1	Serum albumin OS=Mus musculus GN=Alb PE=1 SV=3	628	47	67.27
1	Alpha-1-antitrypsin 1-2 OS=Mus musculus GN=Serpina1b PE=1 SV=2	207	13	24.46
1	Hemoglobin subunit alpha OS=Mus musculus GN=Hba PE=1 SV=2	114	9	83.10
1	Serine protease inhibitor A3K OS=Mus musculus GN=Serpina3k PE=1 SV=2	214	16	39.23
1	Serotransferrin OS=Mus musculus GN=Tf PE=1 SV=1	595	45	60.98
1	Alpha-1-antitrypsin 1-4 OS=Mus musculus GN=Serpina1d PE=2 SV=1	193	15	35.35
1	Apolipoprotein A-I OS=Mus musculus GN=Apoa1 PE=1 SV=2	246	21	45.45
1	Hemoglobin subunit beta-1 OS=Mus musculus GN=Hbb-b1 PE=1 SV=2	99	8	59.18
1	Alpha-1-antitrypsin 1-3 OS=Mus musculus GN=Serpina1c PE=1 SV=2	162	12	25.24
1	Alpha-1-antitrypsin 1-1 OS=Mus musculus GN=Serpina1a PE=1 SV=4	162	12	25.18
1	Serine protease inhibitor A3M OS=Mus musculus GN=Serpina3m PE=1 SV=2	113	7	11.96
1	Alpha-1-antitrypsin 1-5 OS=Mus musculus GN=Serpina1e PE=1 SV=1	157	13	24.94
1	Hemoglobin subunit beta-2 OS=Mus musculus GN=Hbb-b2 PE=1 SV=2	77	6	35.37
1	Alpha-2-macroglobulin OS=Mus musculus GN=A2m PE=1 SV=3	674	52	38.13

(continued on next page)

Table S9. Identified proteins in group treated with 300 mg kg^{-1} day^{-1} of diacetyl (three technical replicates) (cont.)

Replicate number	Protein Description	Protein Matched Products	Protein matched Peptides	Protein seqCover (%)
1	Apolipoprotein A-IV OS=Mus musculus GN=Apoa4 PE=1 SV=3	192	24	64.05
1	Hemopexin OS=Mus musculus GN=Hpx PE=1 SV=2	227	26	57.83
1	Isoform 3 of Kininogen-1 OS=Mus musculus GN=Kng1	162	16	44.79
1	Kininogen-1 OS=Mus musculus GN=Kng1 PE=1 SV=1	166	17	36.31
1	Isoform LMW of Kininogen-1 OS=Mus musculus GN=Kng1	152	15	46.99
1	Carboxylesterase 1C OS=Mus musculus GN=Ces1c PE=1 SV=4	172	16	27.44
1	Alpha-2-HS-glycoprotein OS=Mus musculus GN=Ahsg PE=1 SV=1	97	7	26.96
1	Beta-2-glycoprotein 1 OS=Mus musculus GN=Apoh PE=1 SV=1	99	15	48.12
1	Murinoglobulin-1 OS=Mus musculus GN=Mug1 PE=1 SV=3	384	41	34.82
1	Complement C3 OS=Mus musculus GN=C3 PE=1 SV=3	403	56	40.41
1	Hemoglobin subunit epsilon-Y2 OS=Mus musculus GN=Hbb-y PE=1 SV=2	22	1	6.80
1	Isoform 2 of Fibrinogen alpha chain OS=Mus musculus GN=Fga	122	15	37.34
1	Fibrinogen alpha chain OS=Mus musculus GN=Fga PE=2 SV=1	126	16	33.46
1	Fibrinogen gamma chain OS=Mus musculus GN=Fgg PE=1 SV=1	110	14	37.16
1	Apolipoprotein E OS=Mus musculus GN=Apoe PE=1 SV=2	89	12	39.23
1	Serine protease inhibitor A3N OS=Mus musculus GN=Serpina3n PE=1 SV=1	72	10	24.16
1	Serine protease inhibitor A3G OS=Mus musculus GN=Serpina3g PE=2 SV=2	62	6	10.23
1	Serine protease inhibitor A3C OS=Mus musculus GN=Serpina3c PE=2 SV=1	55	6	8.39
1	Major urinary proteins 11 and 8 (Fragment) OS=Mus musculus GN=Mup8 PE=1 SV=1	45	6	51.66
1	Major urinary protein 17 OS=Mus musculus GN=Mup17 PE=2 SV=2	45	6	43.33
1	Major urinary protein 6 OS=Mus musculus GN=Mup6 PE=1 SV=2	45	6	43.33
1	Murinoglobulin-2 OS=Mus musculus GN=Mug2 PE=2 SV=2	187	20	16.47
1	Fibrinogen beta chain OS=Mus musculus GN=Fgb PE=2 SV=1	160	19	40.54
1	Haptoglobin OS=Mus musculus GN=Hp PE=1 SV=1	83	13	40.35
1	Vitamin D-binding protein OS=Mus musculus GN=Gc PE=1 SV=2	138	18	42.02
1	Ig mu chain C region OS=Mus musculus GN=Ighm PE=1 SV=2	71	8	24.67
1	Isoform 2 of Ig mu chain C region OS=Mus musculus GN=Ighm	70	7	18.95
1	Serine protease inhibitor A3F OS=Mus musculus GN=Serpina3f PE=1 SV=3	51	6	13.48

(continued on next page)

Table S9. Identified proteins in group treated with 300 mg kg^{-1} day^{-1} of diacetyl (three technical replicates) (cont.)

Replicate number	Protein Description	Protein Matched Products	Protein matched Peptides	Protein seqCover (%)
1	Antithrombin-III OS=Mus musculus GN=Serpinc1 PE=1 SV=1	133	17	32.26
1	Major urinary protein 2 OS=Mus musculus GN=Mup2 PE=1 SV=1	35	5	36.67
1	Major urinary protein 1 OS=Mus musculus GN=Mup1 PE=1 SV=1	35	5	36.67
1	Alpha-2-antiplasmin OS=Mus musculus GN=Serpinf2 PE=1 SV=1	63	11	20.16
1	Gelsolin OS=Mus musculus GN=Gsn PE=1 SV=3	140	17	30.13
1	Isoform 2 of Gelsolin OS=Mus musculus GN=Gsn	135	16	31.19
1	Isoform Short of Complement C3 OS=Mus musculus GN=C3	76	15	35.14
1	Plasminogen OS=Mus musculus GN=Plg PE=1 SV=3	168	33	51.23
1	Clusterin OS=Mus musculus GN=Clu PE=1 SV=1	62	10	28.35
1	Apolipoprotein C-III OS=Mus musculus GN=Apoc3 PE=1 SV=2	21	2	27.27
1	Prothrombin OS=Mus musculus GN=F2 PE=1 SV=1	113	17	25.40
1	Fetuin-B OS=Mus musculus GN=Fetub PE=1 SV=1	56	7	25.26
1	Histidine-rich glycoprotein OS=Mus musculus GN=Hrg PE=1 SV=2	69	10	24.95
1	Ig gamma-2A chain C region secreted form OS=Mus musculus PE=1 SV=1	30	3	11.94
1	Plasma protease C1 inhibitor OS=Mus musculus GN=Serping1 PE=1 SV=3	62	12	24.40
1	H-2 class I histocompatibility antigen_ Q10 alpha chain OS=Mus musculus GN=H2-Q10 PE=1 SV=3	37	5	17.85
1	Corticosteroid-binding globulin OS=Mus musculus GN=Serpina6 PE=1 SV=1	28	7	18.89
1	Isoform 2 of Ig gamma-2B chain C region OS=Mus musculus GN=Igh-3	40	7	25.07
1	Ig gamma-2B chain C region OS=Mus musculus GN=Igh-3 PE=1 SV=3	40	7	20.79
1	Carboxylesterase 1D OS=Mus musculus GN=Ces1d PE=1 SV=1	43	5	10.09
1	Ceruloplasmin OS=Mus musculus GN=Cp PE=1 SV=2	129	23	36.19
1	H-2 class I histocompatibility antigen_ Q8 alpha chain OS=Mus musculus GN=H2-Q8 PE=2 SV=1	22	6	27.91
1	H-2 class I histocompatibility antigen_ Q7 alpha chain OS=Mus musculus GN=H2-Q7 PE=1 SV=1	21	5	15.87
1	Isoform 2 of H-2 class I histocompatibility antigen_ alpha chain OS=Mus musculus GN=H2-D1	15	2	7.02
1	H-2 class I histocompatibility antigen_ alpha chain (Fragment) OS=Mus musculus GN=H2-D1 PE=1 SV=1	15	2	6.71
1	H-2 class I histocompatibility antigen_ D-D alpha chain OS=Mus musculus GN=H2-D1 PE=1 SV=1	15	2	5.48
1	H-2 class I histocompatibility antigen_ D-B alpha chain OS=Mus musculus GN=H2-D1 PE=1 SV=2	15	2	5.52

(continued on next page)

Table S9. Identified proteins in group treated with 300 mg kg^{-1} day^{-1} of diacetyl (three technical replicates) (cont.)

Replicate number	Protein Description	Protein Matched Products	Protein matched Peptides	Protein seqCover (%)
1	Beta-2-microglobulin OS=Mus musculus GN=B2m PE=1 SV=2	17	3	21.01
1	Serum paraoxonase/arylesterase 1 OS=Mus musculus GN=Pon1 PE=1 SV=2	37	5	16.06
1	Ig heavy chain V region AC38 205.12 OS=Mus musculus PE=1 SV=1	14	2	27.97
1	Heparin cofactor 2 OS=Mus musculus GN=Serpind1 PE=1 SV=1	29	6	12.55
1	Vitronectin OS=Mus musculus GN=Vtn PE=1 SV=2	23	5	12.55
1	Inter alpha-trypsin inhibitor_ heavy chain 4 OS=Mus musculus GN=Itih4 PE=1 SV=2	92	18	25.16
1	Retinol-binding protein 4 OS=Mus musculus GN=Rbp4 PE=2 SV=2	15	3	20.90
1	Protein AMBP OS=Mus musculus GN=Ambp PE=2 SV=2	21	4	15.19
1	Epidermal growth factor receptor OS=Mus musculus GN=Egfr PE=1 SV=1	82	18	19.92
1	Glutathione peroxidase 3 OS=Mus musculus GN=Gpx3 PE=2 SV=2	24	7	40.27
1	Inter-alpha-trypsin inhibitor heavy chain H2 OS=Mus musculus GN=Itih2 PE=1 SV=1	70	13	17.76
1	Carboxypeptidase N subunit 2 OS=Mus musculus GN=Cpn2 PE=1 SV=2	53	11	32.18
1	Isoform 2 of Inter alpha-trypsin inhibitor_ heavy chain 4 OS=Mus musculus GN=Itih4	80	17	24.70
1	Complement factor H OS=Mus musculus GN=Cfh PE=1 SV=2	99	24	26.90
1	Liver carboxylesterase 1 OS=Mus musculus GN=Ces1 PE=2 SV=1	21	2	2.83
1	Mannose-binding protein C OS=Mus musculus GN=Mbl2 PE=2 SV=2	20	3	12.70
1	Carboxypeptidase N catalytic chain OS=Mus musculus GN=Cpn1 PE=2 SV=1	12	4	10.72
1	Phosphatidylinositol-glycan-specific phospholipase D OS=Mus musculus GN=Gpld1 PE=1 SV=1	57	13	16.61
1	Complement factor B OS=Mus musculus GN=Cfb PE=1 SV=2	76	17	25.49
1	Isoform 3 of Afamin OS=Mus musculus GN=Afm	42	10	20.79
1	Afamin OS=Mus musculus GN=Afm PE=1 SV=2	42	10	20.89
1	Isoform 2 of Afamin OS=Mus musculus GN=Afm	38	7	16.74
1	Zinc-alpha-2-glycoprotein OS=Mus musculus GN=Azgp1 PE=1 SV=2	36	8	26.38
1	Reversed Sequence 12334	18	4	15.48
1	Carbonic anhydrase 1 OS=Mus musculus GN=Ca1 PE=2 SV=4	9	3	27.59
1	Peroxiredoxin-2 OS=Mus musculus GN=Prdx2 PE=1 SV=3	13	4	34.34
1	Fibronectin OS=Mus musculus GN=Fn1 PE=1 SV=4	115	28	16.51

(continued on next page)

Table S9. Identified proteins in group treated with 300 mg kg^{-1} day^{-1} of diacetyl (three technical replicates) (cont.)

Replicate number	Protein Description	Protein Matched Products	Protein matched Peptides	Protein seqCover (%)
1	Angiotensinogen OS=Mus musculus GN=Agt PE=1 SV=1	21	3	7.34
1	Lumican OS=Mus musculus GN=Lum PE=1 SV=2	24	8	16.86
1	Complement factor D OS=Mus musculus GN=Cfd PE=1 SV=1	9	4	29.34
1	Complement C5 OS=Mus musculus GN=C5 PE=1 SV=2	99	18	12.14
1	Interleukin-1 receptor accessory protein OS=Mus musculus GN=Il1rap PE=1 SV=1	29	8	15.61
1	Isoform 2 of Interleukin-1 receptor accessory protein OS=Mus musculus GN=Il1rap	26	6	18.33
1	Complement component C9 OS=Mus musculus GN=C9 PE=1 SV=2	46	11	16.06
1	Inter-alpha-trypsin inhibitor heavy chain H1 OS=Mus musculus GN=Itih1 PE=1 SV=2	45	12	18.96
1	Major urinary protein 5 OS=Mus musculus GN=Mup5 PE=2 SV=1	14	4	31.67
1	Complement component C8 alpha chain OS=Mus musculus GN=C8a PE=2 SV=1	34	11	23.34
1	Isoform 2 of Ig gamma-3 chain C region OS=Mus musculus	16	5	31.31
1	Ig gamma-3 chain C region OS=Mus musculus PE=1 SV=2	16	5	25.88
1	Alpha-2-macroglobulin-P OS=Mus musculus GN=A2mp PE=2 SV=2	28	12	11.19
1	Coagulation factor X OS=Mus musculus GN=F10 PE=1 SV=1	22	9	19.13
1	Apolipoprotein C-I OS=Mus musculus GN=Apoc1 PE=1 SV=1	8	2	20.45
1	Plasma kallikrein OS=Mus musculus GN=Klkb1 PE=1 SV=2	27	9	14.42
1	Isoform 3 of Sulfhydryl oxidase 1 OS=Mus musculus GN=Qsox1	29	8	9.86
1	Isoform 2 of Sulfhydryl oxidase 1 OS=Mus musculus GN=Qsox1	37	10	17.40
1	Sulfhydryl oxidase 1 OS=Mus musculus GN=Qsox1 PE=1 SV=1	37	10	15.37
1	Complement C4-B OS=Mus musculus GN=C4b PE=1 SV=3	74	21	15.25
1	Isoform 2 of Complement component C8 beta chain OS=Mus musculus GN=C8b	25	9	25.62
1	Complement component C8 beta chain OS=Mus musculus GN=C8b PE=1 SV=1	25	9	22.75
1	Coagulation factor XII OS=Mus musculus GN=F12 PE=2 SV=2	36	11	27.14
1	Inhibitor of carbonic anhydrase OS=Mus musculus GN=Ica PE=1 SV=1	38	15	29.14
1	Reversed Sequence 475	21	7	16.36
1	Insulin-like growth factor-binding protein complex acid labile subunit OS=Mus musculus GN=Igfals PE=2 SV=1	20	4	12.60
1	Immunoglobulin J chain OS=Mus musculus GN=Igj PE=2 SV=4	14	5	30.19

(continued on next page)

Table S9. Identified proteins in group treated with 300 mg kg^{-1} day^{-1} of diacetyl (three technical replicates) (cont.)

Replicate number	Protein Description	Protein Matched Products	Protein matched Peptides	Protein seqCover (%)
1	Inter-alpha-trypsin inhibitor heavy chain H3 OS=Mus musculus GN=Itih3 PE=1 SV=3	38	12	15.86
1	Major urinary protein 20 OS=Mus musculus GN=Mup20 PE=1 SV=1	14	4	29.28
1	Extracellular matrix protein 1 OS=Mus musculus GN=Ecm1 PE=1 SV=2	17	8	15.21
1	Major urinary protein 3 OS=Mus musculus GN=Mup3 PE=1 SV=1	19	4	16.85
1	Apolipoprotein D OS=Mus musculus GN=Apod PE=2 SV=1	6	3	12.70
1	Hepatocyte growth factor activator OS=Mus musculus GN=Hgfac PE=1 SV=1	19	6	14.70
1	Complement factor I OS=Mus musculus GN=Cfi PE=1 SV=3	30	8	9.78
1	Leukemia inhibitory factor receptor OS=Mus musculus GN=Lifr PE=1 SV=1	34	13	12.73
1	Isoform 2 of Leukemia inhibitory factor receptor OS=Mus musculus GN=Lifr	32	12	16.55
1	Reversed Sequence 12002	9	4	20.31
1	Reversed Sequence 15257	25	5	25.73
1	Properdin OS=Mus musculus GN=Cfp PE=2 SV=2	16	7	22.63
1	H-2 class I histocompatibility antigen_ Q9 alpha chain (Fragment) OS=Mus musculus GN=H2-Q9 PE=3 SV=1	11	4	11.50
1	Isoform 2 of Protein Z-dependent protease inhibitor OS=Mus musculus GN=Serpina10	16	6	16.24
1	Protein Z-dependent protease inhibitor OS=Mus musculus GN=Serpina10 PE=1 SV=1	17	7	16.29
1	Isoform Short of Extracellular matrix protein 1 OS=Mus musculus GN=Ecm1	12	6	11.29
1	Pigment epithelium-derived factor OS=Mus musculus GN=Serpinf1 PE=1 SV=2	10	4	12.71
1	H-2 class I histocompatibility antigen_ K-D alpha chain OS=Mus musculus GN=H2-K1 PE=1 SV=1	5	1	2.72
1	Pyrethroid hydrolase Ces2e OS=Mus musculus GN=Ces2e PE=1 SV=1	12	5	8.23
1	Mdm2-binding protein OS=Mus musculus GN=Mtbp PE=1 SV=1	27	8	19.24
1	Reversed Sequence 12416	27	12	13.55
1	Keratin_ type I cuticular Ha5 OS=Mus musculus GN=Krt35 PE=2 SV=1	16	9	33.63
1	Carboxylesterase 1E OS=Mus musculus GN=Ces1e PE=1 SV=1	8	1	1.25
1	Ig gamma-1 chain C region secreted form OS=Mus musculus GN=Ighg1 PE=1 SV=1	11	3	14.51
1	Ig gamma-1 chain C region_ membrane-bound form OS=Mus musculus GN=Ighg1 PE=1 SV=2	11	3	11.96

(continued on next page)

Table S9. Identified proteins in group treated with 300 mg kg^{-1} day^{-1} of diacetyl (three technical replicates) (cont.)

Replicate number	Protein Description	Protein Matched Products	Protein matched Peptides	Protein seqCover (%)
1	DNA replication licensing factor MCM2 OS=Mus musculus GN=Mcm2 PE=1 SV=3	35	11	16.81
1	WNT1-inducible-signaling pathway protein 3 OS=Mus musculus GN=Wisp3 PE=3 SV=2	12	6	16.95
1	Reversed Sequence 6465	8	6	15.88
1	SUN domain-containing protein 3 OS=Mus musculus GN=Sun3 PE=2 SV=1	6	2	10.31
1	ER membrane protein complex subunit 9 OS=Mus musculus GN=Emc9 PE=2 SV=1	8	3	10.68
1	Glycerophosphodiester phosphodiesterase domain-containing protein 1 OS=Mus musculus GN=Gdpd1 PE=2 SV=1	7	4	14.33
1	Lymphocyte transmembrane adapter 1 OS=Mus musculus GN=Lax1 PE=2 SV=2	13	5	25.06
1	Isoform 2 of N-acetylmuramoyl-L-alanine amidase OS=Mus musculus GN=Pglyrp2	12	4	10.18
1	N-acetylmuramoyl-L-alanine amidase OS=Mus musculus GN=Pglyrp2 PE=1 SV=1	12	4	9.62
1	TATA element modulatory factor OS=Mus musculus GN=Tmf1 PE=1 SV=2	27	11	10.27
1	Reversed Sequence 20212	11	3	17.17
1	Ataxin-1 OS=Mus musculus GN=Atxn1 PE=1 SV=2	14	5	10.75
1	Isoform 3 of N-acetylmuramoyl-L-alanine amidase OS=Mus musculus GN=Pglyrp2	10	3	11.33
1	Reversed Sequence 817	21	11	22.04
1	Isoform 2 of Elongator complex protein 2 OS=Mus musculus GN=Elp2	17	7	12.42
1	Elongator complex protein 2 OS=Mus musculus GN=Elp2 PE=1 SV=1	18	8	11.07
1	Homeobox protein Hox-D9 OS=Mus musculus GN=Hoxd9 PE=2 SV=1	8	2	11.50
1	WD repeat-containing protein 93 OS=Mus musculus GN=Wdr93 PE=2 SV=1	10	3	2.30
1	Rho-related BTB domain-containing protein 1 OS=Mus musculus GN=Rhobtb1 PE=1 SV=2	11	4	11.94
2	Serum albumin OS=Mus musculus GN=Alb PE=1 SV=3	661	53	59.7
2	Apolipoprotein A-I OS=Mus musculus GN=Apoa1 PE=1 SV=2	179	19	34.47
2	Hemoglobin subunit beta-1 OS=Mus musculus GN=Hbb-b1 PE=1 SV=2	83	8	59.18
2	Hemoglobin subunit alpha OS=Mus musculus GN=Hba PE=1 SV=2	94	8	55.63
2	Serotransferrin OS=Mus musculus GN=Tf PE=1 SV=1	509	46	51.22
2	Alpha-2-macroglobulin OS=Mus musculus GN=A2m PE=1 SV=3	568	55	35.79

(continued on next page)

Table S9. Identified proteins in group treated with 300 mg kg^{-1} day^{-1} of diacetyl (three technical replicates) (cont.)

Replicate number	Protein Description	Protein Matched Products	Protein matched Peptides	Protein seqCover (%)
2	Hemoglobin subunit beta-2 OS=Mus musculus GN=Hbb-b2 PE=1 SV=2	66	6	35.37
2	Serine protease inhibitor A3K OS=Mus musculus GN=Serpina3k PE=1 SV=2	174	14	31.1
2	Alpha-1-antitrypsin 1-5 OS=Mus musculus GN=Serpina1e PE=1 SV=1	132	13	32.45
2	Hemoglobin subunit epsilon-Y2 OS=Mus musculus GN=Hbb-y PE=1 SV=2	29	2	6.8
2	Alpha-1-antitrypsin 1-4 OS=Mus musculus GN=Serpina1d PE=2 SV=1	218	22	44.31
2	Alpha-1-antitrypsin 1-3 OS=Mus musculus GN=Serpina1c PE=1 SV=2	197	20	41.02
2	Alpha-1-antitrypsin 1-1 OS=Mus musculus GN=Serpina1a PE=1 SV=4	197	20	40.92
2	Serine protease inhibitor A3M OS=Mus musculus GN=Serpina3m PE=1 SV=2	81	8	16.03
2	Alpha-1-antitrypsin 1-2 OS=Mus musculus GN=Serpina1b PE=1 SV=2	193	18	44.79
2	Enolase 1 OS=Saccharomyces cerevisiae (st	132	18	42.56
2	Complement C3 OS=Mus musculus GN=C3 PE=1 SV=3	211	32	21.35
2	Isoform 3 of Kininogen-1 OS=Mus musculus GN=Kng1	75	9	27.29
2	Kininogen-1 OS=Mus musculus GN=Kng1 PE=1 SV=1	75	9	19.82
2	Isoform LMW of Kininogen-1 OS=Mus musculus GN=Kng1	72	8	27.55
2	Carboxylesterase 1C OS=Mus musculus GN=Ces1c PE=1 SV=4	108	16	40.97
2	Murinoglobulin-1 OS=Mus musculus GN=Mug1 PE=1 SV=3	237	32	25.54
2	Alpha-2-HS-glycoprotein OS=Mus musculus GN=Ahsg PE=1 SV=1	36	3	8.12
2	Apolipoprotein A-IV OS=Mus musculus GN=Apoa4 PE=1 SV=3	61	12	27.34
2	Hemopexin OS=Mus musculus GN=Hpx PE=1 SV=2	101	15	38.04
2	Apolipoprotein E OS=Mus musculus GN=Apoe PE=1 SV=2	39	6	23.15
2	Apolipoprotein A-II OS=Mus musculus GN=Apoa2 PE=1 SV=2	33	3	14.71
2	Isoform 2 of Fibrinogen alpha chain OS=Mus musculus GN=Fga	82	17	39.5
2	Fibrinogen alpha chain OS=Mus musculus GN=Fga PE=2 SV=1	82	17	27.88
2	Murinoglobulin-2 OS=Mus musculus GN=Mug2 PE=2 SV=2	140	19	11.92
2	Beta-2-glycoprotein 1 OS=Mus musculus GN=Apoh PE=1 SV=1	44	10	33.91
2	Alpha-2-antiplasmin OS=Mus musculus GN=Serpinf2 PE=1 SV=1	46	8	17.31
2	Carboxylesterase 1D OS=Mus musculus GN=Ces1d PE=1 SV=1	36	4	10.27
2	Histidine-rich glycoprotein OS=Mus musculus GN=Hrg PE=1 SV=2	38	7	14.67

(continued on next page)

Table S9. Identified proteins in group treated with 300 mg kg^{-1} day^{-1} of diacetyl (three technical replicates) (cont.)

Replicate number	Protein Description	Protein Matched Products	Protein matched Peptides	Protein seqCover (%)
2	Liver carboxylesterase 1 OS=Mus musculus GN=Ces1 PE=2 SV=1	24	4	11.33
2	Clusterin OS=Mus musculus GN=Clu PE=1 SV=1	54	8	41.96
2	Ig heavy chain V region AC38 205.12 OS=Mus musculus PE=1 SV=1	8	1	16.1
2	Ig heavy chain V region J558 OS=Mus musculus PE=1 SV=1	8	1	16.24
2	Ig heavy chain V region MOPC 104E OS=Mus musculus PE=1 SV=1	8	1	16.24
2	Antithrombin-III OS=Mus musculus GN=Serpinc1 PE=1 SV=1	68	15	34.41
2	Serum paraoxonase/arylesterase 1 OS=Mus musculus GN=Pon1 PE=1 SV=2	16	2	8.45
2	Fibrinogen gamma chain OS=Mus musculus GN=Fgg PE=1 SV=1	38	6	19.04
2	Isoform Short of Complement C3 OS=Mus musculus GN=C3	34	6	14.39
2	Serine protease inhibitor A3C OS=Mus musculus GN=Serpina3c PE=2 SV=1	19	3	3.36
2	Serine protease inhibitor A3N OS=Mus musculus GN=Serpina3n PE=1 SV=1	19	3	3.35
2	Serine protease inhibitor A3G OS=Mus musculus GN=Serpina3g PE=2 SV=2	19	3	3.18
2	Serine protease inhibitor A3F OS=Mus musculus GN=Serpina3f PE=1 SV=3	23	4	10.34
2	Vitamin D-binding protein OS=Mus musculus GN=Gc PE=1 SV=2	50	9	19.33
2	Succinate dehydrogenase [ubiquinone] cytochrome b small subunit_ mitochondrial OS=Mus musculus GN=Sdhd PE=2 SV=2	9	2	15.09
2	Fibrinogen beta chain OS=Mus musculus GN=Fgb PE=2 SV=1	65	14	30.35
2	Isoform 2 of Ig mu chain C region OS=Mus musculus GN=Ighm	74	14	29.47
2	Ig mu chain C region OS=Mus musculus GN=Ighm PE=1 SV=2	74	14	30.84
2	Ceruloplasmin OS=Mus musculus GN=Cp PE=1 SV=2	97	19	23.19
2	Haptoglobin OS=Mus musculus GN=Hp PE=1 SV=1	31	8	22.77
2	Ig kappa chain V-III region PC 6684 OS=Mus musculus PE=1 SV=1	10	1	16.22
2	Ig kappa chain V-III region PC 7769 OS=Mus musculus PE=1 SV=1	10	1	16.22
2	Ig kappa chain V-III region PC 7175 OS=Mus musculus PE=1 SV=1	10	1	16.22
2	Ig kappa chain V-III region PC 2485/PC 4039 OS=Mus musculus PE=1 SV=1	10	1	16.22
2	Ig kappa chain V-III region PC 7940 OS=Mus musculus PE=1 SV=1	10	1	16.22
2	Ig kappa chain V-III region PC 7183 OS=Mus musculus PE=1 SV=1	10	1	16.22
2	Ig kappa chain V-III region PC 7210 OS=Mus musculus PE=1 SV=1	10	1	16.36
2	Ig kappa chain V-III region PC 6308 OS=Mus musculus PE=1 SV=1	10	1	16.22

(continued on next page)

Table S9. Identified proteins in group treated with 300 mg kg^{-1} day^{-1} of diacetyl (three technical replicates) (cont.)

Replicate number	Protein Description	Protein Matched Products	Protein matched Peptides	Protein seqCover (%)
2	Ig kappa chain V-III region PC 7043 OS=Mus musculus PE=1 SV=1	10	1	16.22
2	Ig kappa chain V-III region CBPC 101 OS=Mus musculus PE=1 SV=1	10	1	16.22
2	Ig kappa chain V-III region PC 3741/TEPC 111 OS=Mus musculus PE=1 SV=1	10	1	16.22
2	Ig kappa chain V-III region TEPC 124 OS=Mus musculus PE=1 SV=1	10	1	16.07
2	Ig kappa chain V-III region MOPC 70 OS=Mus musculus PE=1 SV=1	10	1	16.22
2	Ig kappa chain V-III region PC 7132 OS=Mus musculus PE=1 SV=1	10	1	16.07
2	Ig kappa chain V-III region PC 2413 OS=Mus musculus PE=1 SV=1	10	1	16.22
2	Ig kappa chain V-III region 50S10.1 OS=Mus musculus PE=1 SV=1	10	1	16.22
2	Ig kappa chain V-III region PC 2880/PC 1229 OS=Mus musculus PE=1 SV=1	10	1	16.22
2	Beta-2-microglobulin OS=Mus musculus GN=B2m PE=1 SV=2	13	3	19.33
2	Reversed Sequence 3017	27	4	19.5
2	Alpha-2-macroglobulin-P OS=Mus musculus GN=A2mp PE=2 SV=2	33	11	11.19
2	Complement factor B OS=Mus musculus GN=Cfb PE=1 SV=2	49	13	20.5
2	Regenerating islet-derived protein 3-alpha OS=Mus musculus GN=Reg3a PE=1 SV=1	9	2	27.43
2	Complement component C9 OS=Mus musculus GN=C9 PE=1 SV=2	42	11	20.8
2	Inter-alpha-trypsin inhibitor heavy chain H2 OS=Mus musculus GN=Itih2 PE=1 SV=1	48	13	19.56
2	Prothrombin OS=Mus musculus GN=F2 PE=1 SV=1	41	7	14.56
2	Plasminogen OS=Mus musculus GN=Plg PE=1 SV=3	51	13	26.85
2	Nucleoprotein (Fragment) OS=Sendai virus (strain Harris) GN=N PE=1 SV=1	9	3	33.73
2	Plasma protease C1 inhibitor OS=Mus musculus GN=Serping1 PE=1 SV=3	14	2	5.56
2	Isoform 2 of Ig gamma-2B chain C region OS=Mus musculus GN=Igh-3	16	4	16.12
2	Ig gamma-2B chain C region OS=Mus musculus GN=Igh-3 PE=1 SV=3	16	4	13.37
2	Gelsolin OS=Mus musculus GN=Gsn PE=1 SV=3	48	14	17.95
2	Isoform 2 of Gelsolin OS=Mus musculus GN=Gsn	46	13	18.19
2	Carboxypeptidase N catalytic chain OS=Mus musculus GN=Cpn1 PE=2 SV=1	8	3	8.97
2	Isoform 2 of Interleukin-1 receptor accessory protein OS=Mus musculus GN=Il1rap	15	5	13.89

(continued on next page)

Table S9. Identified proteins in group treated with 300 mg kg^{-1} day^{-1} of diacetyl (three technical replicates) (cont.)

Replicate number	Protein Description	Protein Matched Products	Protein matched Peptides	Protein seqCover (%)
2	Interleukin-1 receptor accessory protein OS=Mus musculus GN=Il1rap PE=1 SV=1	15	5	8.77
2	Reversed Sequence 431	15	6	15.55
2	H-2 class I histocompatibility antigen_ Q10 alpha chain OS=Mus musculus GN=H2-Q10 PE=1 SV=3	15	3	12.92
2	RNA-directed RNA polymerase L OS=Lymphocytic choriomeningitis virus (strain Armstrong) GN=L PE=1 SV=1	44	16	9.95
2	Complement factor H OS=Mus musculus GN=Cfh PE=1 SV=2	52	14	15.72
2	Plasma kallikrein OS=Mus musculus GN=Klkb1 PE=1 SV=2	24	7	11.13
2	Isoform 2 of Inter alpha-trypsin inhibitor_ heavy chain 4 OS=Mus musculus GN=Itih4	37	9	9.75
2	Inter alpha-trypsin inhibitor_ heavy chain 4 OS=Mus musculus GN=Itih4 PE=1 SV=2	37	9	9.34
2	Peroxiredoxin-2 OS=Mus musculus GN=Prdx2 PE=1 SV=3	9	2	9.09
2	Angiotensinogen OS=Mus musculus GN=Agt PE=1 SV=1	15	5	17.61
2	Apolipoprotein C-III OS=Mus musculus GN=Apoc3 PE=1 SV=2	4	1	19.19
2	Reversed Sequence 16327	8	4	15.5
2	Glycerophosphodiester phosphodiesterase 1 OS=Mus musculus GN=Gde1 PE=2 SV=1	6	1	3.93
2	M-phase inducer phosphatase 1 OS=Mus musculus GN=Cdc25a PE=2 SV=2	13	5	11.48
2	Reversed Sequence 6339	13	6	20.03
2	Anoctamin-4 OS=Mus musculus GN=Ano4 PE=2 SV=2	17	9	12.04
2	Mannose-binding protein C OS=Mus musculus GN=Mbl2 PE=2 SV=2	9	4	9.43
2	Complement component C8 gamma chain OS=Mus musculus GN=C8g PE=1 SV=1	5	2	10.4
2	Isoform 2 of Plakophilin-4 OS=Mus musculus GN=Pkp4	31	8	4.97
2	Plakophilin-4 OS=Mus musculus GN=Pkp4 PE=1 SV=1	31	8	4.79
2	Isoform 2 of Transcription factor Sp9 OS=Mus musculus GN=Sp9	11	1	4.51
2	Transcription factor Sp9 OS=Mus musculus GN=Sp9 PE=2 SV=1	11	1	4.34
2	Isoform 2 of Complement component C8 beta chain OS=Mus musculus GN=C8b	12	2	5.54
2	Complement component C8 beta chain OS=Mus musculus GN=C8b PE=1 SV=1	12	2	4.92