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Original Research Article

Taurine drives body protein renewal and accretion in beef steers

Shuo Zhang [†], Jinming Hu [†], Yufeng Liu, Xu Shen, Cheng Liu, Long Cheng, Mengmeng Li, Guangyong Zhao [*]

State Key Laboratory of Animal Nutrition and Feeding, College of Animal Science and Technology, China Agricultural University, Beijing 100193, China

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ABSTRACT

The aims of the present study were to investigate the effects of dietary supplementation with rumen-protected taurine (RPT) on the whole-body protein turnover, the plasma metabolomics, and the whole blood cell transcriptomics in steers. Eight steers, averaging 220 ± 3.26 kg of liveweight, were allocated in a replicate 4 × 4 Latin square design. The experimental treatments consisted of four levels of RPT supplementation: 0, 25, 50, and 75 g RPT per day, added to a basal diet. The results showed that supplementation with RPT linearly decreased the fecal nitrogen (N) excretion ($P = 0.001$) and the ^{15}N fractional recovery rate ($P = 0.047$), while it linearly increased the urinary excretion of taurine ($P = 0.045$) as well as the average daily weight gain ($P = 0.003$), the protein synthesis ($P < 0.001$), the protein degradation ($P < 0.001$) and the whole-body protein turnover ($P < 0.001$). Supplementation with RPT linearly increased the plasma concentrations of growth hormone ($P = 0.005$) and quadratically affected the plasma concentration of insulin-like growth factor-1 ($P = 0.013$), and it linearly decreased the plasma concentration of albumin ($P = 0.022$). Supplementation with RPT altered the whole blood cell mRNA expression and upregulated the expressions of the marker genes, including *RPS6KB1*, *PRSS42*, *COL1A2*, *ENSBTAG00000013055* and *ENSBTAG00000038159* which are related to protein metabolism. The plasma metabolomics profiling indicated that supplementation with RPT upregulated the plasma concentrations of taurine, lysine and methionine. The experiment revealed the impact and the mechanisms of taurine on driving whole-body protein turnover and protein accretion in steers. Two novel marker genes which could be related to body protein degradation in steers were identified.

1. Introduction

Whole-body protein turnover and protein accretion in animals are integrated results of body protein breakdown and re-synthesis, affecting animal health (Hipp et al., 2019) and productive performance (Vignale et al., 2018; Esmaeili et al., 2021). Methionine (Met) is an essential amino acid (AA) for protein synthesis in mammals. Supplementing with adequate Met improved protein synthesis and accretion (Zhao et al., 2020). A major part of the absorbed Met can be directly utilized for protein synthesis, while another part of Met can be used for taurine synthesis in the liver (Lourenco and Camilo, 2002).

Taurine, namely 2-aminoethanesulfonic acid, is a sulfur-containing β-AA (Schaffer et al., 2014). It is one of the most abundant AA in animals, accounting for approximately 0.1 % of animal's body weight and existing throughout tissues and body fluids in a free state (Kim et al., 2014). However, taurine is neither a component of body proteins nor a precursor for synthesizing body proteins (Merckx and De Paepe, 2022). Animal-origin feeds and seafoods contain high levels of taurine, whereas plant-origin feeds hardly contain any taurine (Schaffer et al., 2014). Cattle are herbivores with no animal-origin feeds in their diets. The taurine supply for cattle comes entirely from their own synthesis using Met as the main precursor, consuming a considerable amount of Met which can be directly used for body protein synthesis. This could be an important reason why ruminants, especially beef cattle, have a

* Corresponding author.
E-mail address: zhaogy@cau.edu.cn (G. Zhao).
† Equal contributors.
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lower nitrogen (N) utilization efficiency (NUE) than other species of animals such as swine and poultry (Huhtanen and Hristov, 2009). Taurine can be synthesized endogenously in the liver of most mammals. However, this is insufficient to meet the requirements of mammals (García-Ayuso et al., 2023), and cattle should usually be in a status of taurine deficiency. Therefore, supplementation with taurine should improve the NUE and the body protein turnover in steers.

The aim of this experiment was to study the effects of dietary supplementation with taurine on the NUE and the whole-body protein turnover in steers and further investigate the mechanisms of action through profiling of the plasma metabolomics and whole-blood cell gene transcriptomics.

2. Materials and methods

2.1. Animal ethics statement

The procedures of the experiment were approved by the Animal Care Advisory Committee of China Agricultural University (No. AW82803202-1-1).

2.2. Animals and experimental design

Eight Simmental steers (approximately six months old), with an initial liveweight of 220 ± 3.26 kg, were used as experimental animals. Four levels of rumen-protected taurine (RPT; purity ≥ 60%, Hangzhou King Techina Feed Co., Ltd., China), i.e. 0 (Control), 25, 50, 75 g RPT/d were added to a basal diet (Table 1) as treatments respectively. The in vitro rumen bypass rate and the small intestine digestibility of RPT were 87.34% and 82.96%, respectively, which were determined using the two-stage digestion technique of Tilley and Terry (1963). The animals and the treatments were assigned in a replicated 4 × 4 Latin square design. Each experimental period included 20 d, of which 15 d were for adaptation and 5 d were for sampling.

The animals were tethered and housed in individual pens equipped with feed troughs and had free access to fresh drinking water during the experiment. The basal diet was formulated according to the Nutrient Requirements and Feeding Standards of Beef Cattle (Feng, 2000). The animals were on restricted feeding to minimize the effects of feed intake. Each animal was supplied with 3.54 kg dry matter (DM) in the form of total mixed ration (TMR), which was approximately 90% of the ad libitum DM intake determined in a pre-trial. The daily allowances of diet and RPT were divided into two equal portions and provided to each animal at 07:00 and 16:00, respectively. The RPT was well mixed with the diet before feeding. No orts from the animals were left during the whole experiment. The liveweight of each animal was recorded before morning feeding at the beginning and the end of each experimental period. The feeding and management of the animals were kept stable throughout the experiment.

Table 1
Ingredients and nutritional composition of basal diet (%, DM basis).

Item	Contents
Ingredients	
Corn silage	56.00
Corn grain	12.76
Soybean meal	8.80
Corn gluten feed	9.68
Wheat bran	8.80
NaCl	1.10
$Ca(HCO_3)_2$	1.76
$NaHCO_3$	1.10
Nutritional composition	
OM	97.90
CP	13.59
NDF	44.16
ADF	21.78
GE, MJ/kg	15.66
NE_{mf}[1], MJ/kg	4.99

ADF = acid detergent fiber; CP = crude protein; DM = dry matter; GE = gross energy; NDF = neutral detergent fiber; NE_{mf} = net energy for maintenance and fattening; OM = organic matter.
[1] NE_{mf} was calculated based on dietary GE, OM and NDF contents according to the Nutrient Requirements and Feeding Standards of Beef Cattle (Feng, 2000).

2.3. Urine and feces sampling and isotopic N switch

During each sampling period, the urine from each animal was collected using a rubber funnel attached to the ventral portion of the abdomen connected to a plastic container via a plastic tube. Each urine container had 300 mL (20%, v/v) H_2SO_4 added in advance to keep the urine pH < 3.0. Each urine container was surrounded by ice packs to prevent N loss. The daily output of the urine from each steer was recorded and homogenized, with 3% of the total urine being taken as the sample.

On the second day of each experimental period before feeding in the morning, 5 mL of ^{15}N glycine (abundance ≥ 99 atom%, purity ≥ 98%, Aladdin Biochemical Technology Co., Ltd., Shanghai, China) solution containing 660 mg ^{15}N glycine (concentration 132 mg/mL, made up by dissolving 5.28 g ^{15}N glycine in 40 mL physiological saline), was filtered through a Millipore filtration system (0.45 μmol/L; Millipore, Bedford, MA, USA) before being injected into the jugular vein of each steer.

During each sampling period, the daily feces from each animal was also collected and the amount of the feces was recorded, with 1% of the total feces being taken as the sample, which was mixed with 15 mL H_2SO_4 (20%, v/v) to prevent N loss. Feed samples were also collected daily during each sampling period. The samples of urine, feces and feeds were stored in a refrigerator at −20 °C before chemical analyses.

On the last day of each sampling period, before morning feeding, a 10-mL blood sample was taken from each steer through the jugular vein using a tube containing anticoagulant (Greiner Bio-one, Frickenhausen, Germany) and was centrifuged at 20,000 × *g* for 15 min at 4 °C to obtain the plasma. Subsequently, another aliquot of 10 mL blood sample was taken from each animal into a tube with anticoagulant and TRIzol reagent (Invitrogen, USA) (blood: TRIzol = 1:3, v/v) and homogenized to prevent RNA degradation. The samples were promptly stored in liquid N.

2.4. Chemical analyses

The samples of silage and feces were lyophilized using a freeze dryer (LGJ-12; Songyuan Huaxing Technology Development Co., Ltd., Beijing, China) for 96 h. The fecal samples were ground using a mortar and a pestle, and the silage samples were ground using a grinder (FW177, Tianjin Taisite Instrument Co., Ltd., Tianjin, China) and filtered through a sieve of 40 mesh in pore size.

The DM and ash of feeds and feces were analyzed according to AOAC (2005) using methods 930.15 and 942.05, respectively. The organic matter (OM) was calculated as DM minus crude ash. The neutral detergent fiber (NDF) and acid detergent fiber (ADF) of feeds and feces were analyzed according to the methods described by Van Soest et al. (1991) using an ANKOM 2000 Fiber Analyzer (Ankom Technology Corp., Macedon, NY, USA) with Na_2SO_4 and heat-stable α-amylase in NDF analysis. The total N of

the feeds, feces, and urine was analyzed using the Kjeldahl method according to AOAC (2005) using method 981.10. The crude protein (CP) content was calculated as N multiplied by 6.25. The gross energy (GE) was analyzed using an automatic bomb calorimeter (Parr 6300 Calorimeter; Parr Instrument Company, Moline, IL, USA).

The urinary urea was isolated according to the methods described by Ferguson et al. (2023), and the ^{15}N enrichment in urinary urea was determined using an isotope ratio mass spectrometer coupled with a vario PYRO cube elemental analyzer (Elementar, Langenselbold, Germany). The urine sample collected on the first day of each sampling period (prior to administering ^{15}N glycine) was used for analyzing the background enrichment of ^{15}N in urinary urea. The cocentration of urinary urea was analyzed using the colorimetric method with commercial kits (Nanjing Jiancheng Bioengineering Institute, Jiangsu, China).

The allantoin and uric acid of urine samples were analyzed using the methods of Chen and Gomes (1992). The creatinine of urine samples was analyzed using the Jaffe's method (Yatzidis, 1974). The analyses were conducted on a spectrophotometer (UV-1801; Beijing BeifenRuili Analytical Instrument Co., Ltd., Beijing, China).

The hippuric acid of urine samples was analyzed according to China National Health Standard (WS/T 52-1996, 1996). Briefly, two aliquots of 0.5 mL diluted urine sample were transferred into two spectrophotometric cuvettes. One cuvette used as the treatment had 0.6 mL quinoline and 0.2 mL benzoyl chloride added, and the other cuvette used as the baseline control had 4.5 mL ethanol added. The cuvettes were sealed and vortexed for 20 s and then incubated in dark at 30 ± 2 °C for 30 min. Afterwards, an aliquot of 3.7 mL ethanol was added into each cuvette. The cuvettes were vortexed and incubated in dark for 30 min. The absorbances of the liquids in the cuvettes were measured at 470 nm on a spectrophotometer (UV-1801, Beijing Beifen-Ruili Analytical Instrument Co., Ltd., China).

2.5. Analysis of taurine in urine

The taurine of urine samples was analyzed on HPLC (LC98-1, Beijing Wenfen Analytical Instrument Technology Development Co., Ltd., Beijing, China) using the method described by Yan et al. (2012). The mobile phase consisted of a solution of acetonitrile (0.05 mol/L) and sodium dihydrogen phosphate (pH = 6.5) (15:85, v:v), with a flow rate of 1.0 mL/min. The column temperature was maintained at 35 °C, and the detection wavelength was at 360 nm. The urine samples and the taurine standard were derivatized using a solution of 0.5% 2,4-dinitrofluorobenzene in acetonitrile at 60 °C for 10 min. Then, the mixture was centrifuged at 20,000 × *g* for 5 min at 4 °C prior to analysis.

2.6. Analyses of plasma biochemical parameters

The total protein (TP), albumin (ALB), urea, triglyceride (TG), total AA and total antioxidant capacity (T-AOC) of plasma samples were analyzed using commercial kits (Sino-UK Institute of Biological Technology Co., Ltd., Beijing, China) on an automatic biochemical analyzer (BS-420; Shenzhen Mindray Biomedical Electronic Co., Ltd., Guangdong, China). The plasma globulin (GLB) was calculated as the difference between TP and ALB. The growth hormone (GH) and insulin-like growth factor-1 (IGF-1) of plasma samples were analyzed using the enzyme-linked immunosorbent assay kits (Sino-UK Institute of Biological Technology Co., Ltd., Beijing, China) on a semiautomatic biochemical analyzer (7170; Hitachi Ltd., Tokyo, Japan).

2.7. Plasma metabolomics profiling

The plasma samples for liquid chromatograph-mass spectrometer (LC–MS) analysis were prepared following the procedures described by Wang et al. (2019). One hundred microliters plasma and 400 μL solution composed of acetonitrile and methanol (1:1, v/v), containing 0.02 mg/mL of the internal standard 2-chloro-L-phenylalanine, were mixed and vortexed for 30 s with ultrasonic extraction for 30 min (5 °C, 40 kHz), and then kept at −20 °C for 30 min. Subsequently, the solution was centrifuged at 13,000 × *g* for 15 min at 4 °C and the supernatant was collected for analysis.

The LC–MS analysis was performed using a Thermo UHPLC-Q Exactive HF-X system equipped with an ACQUITY HSS T3 column. The mobile phases consisted of solvent A [0.1% formic acid in a mixture of water and acetonitrile (95:5, v/v)] and solvent B [0.1% formic acid in a mixture of acetonitrile, isopropanol and water (47.5:47.5:5, v/v/v)]. The mass spectrometric data were recorded using a Thermo UHPLC-Q Exactive HF-X Mass Spectrometer equipped with an electrospray ionization source operating in positive and negative modes. To monitor the stability of the analysis, the quality control (QC) sample was prepared by mixing all extracted plasma samples at the same proportion and injected at regular intervals (every 5 samples). The raw data were preprocessed using Progenesis QI software, excluding internal standards, noise, and other artefacts, then the metabolites were identified via the Human Metabolome Database (HMDB) (https://hmdb.ca) and METLIN databases (https://metlin.scripps.edu). Sample preparation and instrument variability were corrected using sum normalization, excluding QC samples with RSD > 30% and the data were log 10-transformed. Then, the ropls package of the R (Version 4.3.0) was used to perform the principal component analysis (PCA) and the orthogonal least partial squares discriminant analysis (OPLS-DA). The metabolites with variable importance projection (VIP) > 1 and *P*-value < 0.05 were deemed significantly different. The metabolic pathway analysis was performed using the *Bos taurus* pathway library (Kyoto Encyclopedia of Genes and Genomes, KEGG; http://www.kegg.com).

2.8. Whole-blood cell transcriptome

The whole-blood cell RNA was extracted using the PAXgene Blood RNA kit (Qiagen, Germany) following the manufacturer's protocol. The RNA concentration and purity were determined using a NanoDrop One^{C} spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA), ensuring an A260/A280 ratio ranging from 1.8 to 2.0. The RNA integrity numbers of all samples were ensured to be above 8.0. An Illumina mRNA-seq library kit was used to construct a targeted transcriptome library, followed by the assessment of the quality of the cDNA library using the Agilent 2100 system (Agilent Technologies, Palo Alto, CA, USA). Sequencing-confirmed libraries underwent paired-end sequencing on the Illumina HiSeq 2000 Platform, with raw data quality checked by FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). High-quality reads were obtained after removing raw reads containing more than 5% unidentified nucleotides and other reads characterized by diminished quality scores, then aligned to the *B. taurus* ARS-UCD1.2 reference genome using HISAT2 v2.2 (Kim et al., 2015). Alignments were sorted by SAMtools v1.9 (Danecek et al., 2021) and read normalization to fragments per kilobase million and transcripts per million (TPM) were conducted using the package of dplyr of R (version 4.3.0). The DESeq2 (v1.28.1) of R (version 4.3.0) was used for correcting read counts and identifying differentially expressed genes (DEGs) with $|\log_2(\text{fold change})| > 1$ and $P < 0.05$.

The DEGs and other gene sets were subjected to analysis using the Gene Ontology (GO) and the KEGG pathways to uncover the

potential biological functions. The aforementioned enrichment analyses for both GO and KEGG were conducted using the KEGG Orthology-based annotation system (KOBAS, http://kobas.cbi.pku.edu.cn/genelist/) (Bu et al., 2021). The ClueGO analysis was conducted using the Cytoscape App (3.10.1) with a selection of levels 3 to 8 of the GO hierarchy. A gene set was considered to be enriched if the *P*-value associated with the hypergeometric test was lower than 0.05, after applying the Benjamini–Hochberg correction for multiple testing.

2.9. Analysis of the weighted gene co-expression network and the construction of protein–protein interaction network

The top 5000 highly variable genes of the whole-blood cells among different treatments were subjected to the weighted gene co-expression network analysis (WGCNA, v1.70-3) (Langfelder and Horvath, 2008). A soft-thresholding power of 4 was applied to construct the adjacency matrix. The co-expressed genes within the co-expression network were identified as the gene network modules (GNMs) marked with different colors and nine co-expression modules were obtained. The clustering was performed on the 9 co-expression modules with the minimum module size of 50. The gene ontology biological process (GO-BP) terms were also analyzed for all nine modules.

The co-expression analysis showed the relationships between the gene expressions within each module and the protein metabolism parameters. Subsequently, the phenotype-associated GNMs with a significance threshold of $P < 0.05$ were filtered. The marker genes were selected based on their within-module connectivity, and they were screened by the weight value within a single module. The Wolf software (Horton et al., 2005) was used to predict the subcellular localization of the proteins possibly encoded by *ENSBTAG00000013055* and *ENSBTAG00000038159*. The protein–protein interaction (PPI) network was constructed and visualized using the STRING database (https://string-db.org/) (Szklarczyk et al., 2021).

2.10. Calculations and statistical analysis

The N retention and the NUE were calculated as:

$$NR = NI - FN - UN,$$

$$NUE = NR/NI \times 100,$$

where *NR* is the N retention, g/d; *NI*, the N intake, g/d; *FN*, the fecal N, g/d; *UN*, the urinary N, g/d.

The ^{15}N glycine single-dose urea end product method was applied to quantify the whole-body protein turnover (Assimon and Stein, 1992; Wessels et al., 1997). The body's total protein turnover was calculated using the method described by Waterlow et al. (1978) as:

$$Q = d/G,$$

where *Q* is the protein turnover (g N/d); *d*, the rate of urinary urea N excretion (g/d); *G*, the fractional recovery of ^{15}N in urinary urea from ^{15}N glycine (%). The ^{15}N enrichment of each urine sample was corrected by subtracting background ^{15}N enrichment.

The whole-body protein synthesis was calculated as:

$$PS = Q - UN,$$

where *PS* is the protein synthesis (g N/d); *Q* is the protein turnover (g N/d).

The whole-body protein degradation was calculated as:

$$PD = PS - NR,$$

where *PD* is the protein degradation (g N/d).

The data of the experiment were statistically analyzed as a replicated Latin square design using the MIXED procedure in SAS (version 9.3; SAS Institute, Inc., Cary, NC, USA) with the following model:

$$Y_{ijkl} = \mu + T_i + P_j + C_k + S_l + e_{ijkl},$$

where Y_{ijkl} represents the observation of the dependent variable; μ, the mean value of population; T_i, the fixed effect of treatment i; P_j, the fixed effect of period j; C_k, the random effect of cattle k; S_l, the effect of square l; e_{ijkl}, the error associated with the observation. The polynomial analysis was performed using the CONTRAST procedures of SAS. For all statistical analyses, significance was considered at $P < 0.05$, and trends were noted at $0.05 \leq P < 0.10$.

3. Results

3.1. N balance, whole-body protein turnover and ADG

Dietary supplementation with RPT did not affect the urinary N excretion ($P = 0.062$; Table 2), but linearly decreased the fecal N excretion ($P = 0.001$) and the ^{15}N fractional recovery ($P = 0.047$) and linearly increased the urinary excretion of taurine ($P = 0.045$; Table 2). Adding RPT linearly increased the protein synthesis ($P < 0.001$) and degradation ($P < 0.001$; Table 2), resulting in the increased whole-body protein turnover ($P < 0.001$; Table 2). Adding RPT also linearly increased the N retention ($P < 0.001$; Table 2), the NUE ($P < 0.001$; Table 2) and the average daily weight gain (ADG) ($P = 0.003$; Table 2).

3.2. Plasma biochemical parameters

Dietary supplementation with RPT did not influence the plasma concentrations of TP ($P = 0.061$), GLB ($P = 0.512$), TG ($P = 0.100$), urea ($P = 0.971$), and T-AOC ($P = 0.395$; Table 3), but it linearly decreased the plasma concentration of ALB ($P = 0.022$; Table 3). Dietary supplementation with RPT also linearly increased the plasma concentration of GH ($P = 0.005$) and quadratically affected the plasma concentration of IGF-1 ($P = 0.013$).

3.3. Urine nitrogenous compounds

Dietary supplementation with RPT did not affect the urinary excretions of urea ($P = 0.213$), uric acid ($P = 0.985$), allantoin ($P = 0.798$), creatinine ($P = 0.545$), hippuric acid ($P = 0.432$), the total urinary excretions of purine derivatives (uric acid + allantoin) ($P = 0.845$) and the estimated rumen microbial N supply ($P = 0.589$) (Table 4).

3.4. Plasma metabolome

With strict quality control and identification, a total of 844 metabolites and 514 KEGG pathways (Fig. S1A) were detected. The metabolites covered a wide range including lipids (41.97%), organic acids (17.31%), organoheterocyclic compounds (12.33%), organic oxygen compounds (8.59%), benzenoids (7.85%) and other metabolites (Fig. S1B). The metabolites of plasma samples showed clear separations among treatments, which became more pronounced with the increase of RPT dosages (Fig. S1C). The permutation test

Table 2
Effects of rumen-protected taurine (RPT) supplementation on N metabolism and protein turnover in steers.

Item	RPT supplemented, g/d				SEM	*P*-value	
	0	25	50	75		L	Q
DMI, kg/d	3.54	3.54	3.54	3.54	–	–	–
RPT intake, g/d	0.00	25.00	50.00	75.00	–	–	–
ADG, kg/d	0.25	0.35	0.43	0.50	0.041	0.003	0.710
Feed N, g/d	76.92	76.92	76.92	76.92	–	–	–
Taurine N, g/d	0.00	1.68	3.36	5.04	–	–	–
Total N intake, g/d	76.92	78.60	80.28	81.96	–	–	–
Urinary N, g/d	35.42	35.20	35.22	38.86	1.160	0.062	0.116
Fecal N, g/d	20.51	17.42	19.56	15.52	0.713	0.001	0.436
Urinary taurine, mg/d	0.45	0.52	0.55	0.69	0.096	0.045	0.631
^{15}N Fractional recovery, %	7.08	7.22	6.89	6.45	0.281	0.047	0.256
Protein turnover, g N/d	183.12	194.73	198.93	216.70	2.875	<0.001	0.310
Protein synthesis, g N/d	147.57	159.52	163.93	177.76	3.093	<0.001	0.769
Protein degradation, g N/d	127.85	133.01	137.94	147.64	2.792	<0.001	0.471
NR, g N/d	21.10	24.74	25.64	27.34	0.815	<0.001	0.264
NUE, %	27.54	31.53	31.94	34.70	0.919	<0.001	0.502

ADG = average daily weight gain; DMI = dry matter intake; SEM = standard error of the mean; L = Linear; N = nitrogen; NR = nitrogen retention; NUE = nitrogen utilization efficiency; Q = quadratic.

Table 3
Effects of rumen-protected taurine (RPT) supplementation on plasma parameters in steers.

Item	RPT supplemented, g/d				SEM	*P*-value	
	0	25	50	75		L	Q
TP, g/L	70.33	67.02	67.32	64.90	1.807	0.061	0.812
ALB, g/L	27.59	26.10	25.26	24.24	0.977	0.022	0.819
GLB, g/L	42.56	42.07	40.92	40.65	1.547	0.512	0.947
TG, mmol/L	0.20	0.24	0.19	0.18	0.020	0.100	0.174
Urea, mmol/L	3.90	4.32	4.12	3.98	0.222	0.971	0.229
GH, ng/mL	5.76	5.876	6.11	6.68	0.212	0.005	0.303
IGF-1, ng/mL	202.60	196.80	198.10	209.01	3.045	0.156	0.013
T-AOC, U/mL	8.36	8.34	8.21	8.52	0.116	0.395	0.127

ALB = albumin; GH = growth hormone; GLB = globulin; IGF-1 = insulin-like growth factor-1; L = linear; Q = quadratic; SEM = standard error of the mean; T-AOC = total antioxidative capacity; TG = triglyceride; TP = total protein.

revealed that the Q^2 intercept was −0.20 which was lower than 0.00, indicating that the model exhibited no overfitting (Fig. S1D).

The data of plasma metabolites were statistically analyzed based on the VIP values observed from the OPLS-DA analysis. It was considered to be differential when $P < 0.05$ and VIP > 1 for metabolites among treatments. As shown in the volcano plots, the amounts of differential metabolites in plasma samples increased with the RPT dosage.

Fig. S1 shows that supplementing with 25 g RPT/d upregulated 22 and downregulated 12 metabolites (Fig. S1E); 50 g RPT/d upregulated 62 and downregulated 29 metabolites (Fig. S1F); and 75 g RPT/d upregulated 118 and downregulated 43 metabolites in plasma samples (Fig. S1G), respectively. In detail, supplementing with 25 g RPT/d upregulated the plasma concentrations of taurine, arginine, ovalicin, and N-linoleoyl-histidine (Fig. 1A and B); 50 g RPT/d upregulated the plasma concentrations of taurine, 3-indoleacetic acid, L-erythro-4-hydroxyarginine and ovalicin and downregulated the plasma concentrations of glycocholic acid, glycocholenate sulfate, and N-choloylglycine (Fig. 1C and D); and 75 g RPT/d upregulated the plasma concentrations of taurine, lysine, methionine, 3-indoleacetic acid, aminofructose 6-phosphate, ergocornine, 4-(2-chloroanilino)-4-oxobutanoic acid, 4-phenylpiperidine-4-carboxylic acid, L-4-chlorotryptophan, 2-hydroxyphenylacetic acid, and ovalicin (Fig. 1E and F), respectively.

The KEGG topology analysis was used to examine the pathways of different metabolites. It was found that supplementing with RPT enriched the pathway involved in taurine and hypotaurine metabolism. Specifically, supplementing with 25 g RPT/d affected the pathways related to sulfur metabolism, phenylalanine metabolism, and glycerophospholipid metabolism (Fig. 1G); 50 g RPT/d enriched the pathways related to glycerophospholipid metabolism, tyrosine metabolism, tryptophan metabolism, arginine, proline metabolism, cysteine and methionine metabolism (Fig. 1H); while 75 g RPT/d enriched the pathways related to tryptophan metabolism, lysine degradation, tyrosine metabolism, fructose and mannose metabolism, and glycerophospholipid metabolism (Fig. 1I).

3.5. Transcriptomic features of whole-blood cells

The principal component analysis (PCA) (Fig. S2A) demonstrated the distinctions between the control and the RPT treatment

Table 4
Effects of rumen-protected taurine (RPT) supplementation on urinary N compounds and estimated rumen microbial N supply in steers.

Item	RPT supplemented, g/d				SEM	*P*-value	
	0	25	50	75		L	Q
Urea, mmol/d	738.39	801.63	790.74	794.99	32.213	0.213	0.319
Uric acid, mmol/d	6.07	5.80	5.40	6.22	0.524	0.985	0.263
Creatinine, mmol/d	88.07	90.65	85.94	86.06	4.038	0.545	0.762
Allantoin, mmol/d	33.29	30.67	34.48	32.86	2.086	0.798	0.812
Hippuric acid, mmol/d	95.91	111.09	100.82	88.77	9.400	0.432	0.152
Purine derivatives, mmol/d	37.42	37.43	38.88	40.08	2.227	0.845	0.695
Estimated rumen microbial N supply[1], g/d	23.73	24.40	25.28	27.15	2.001	0.598	0.311

L = Linear; N = nitrogen; Q = quadratic; RPT = rumen-protected taurine; SEM = standard error of the mean.
[1] Estimated according to Chen and Gomes (1992).

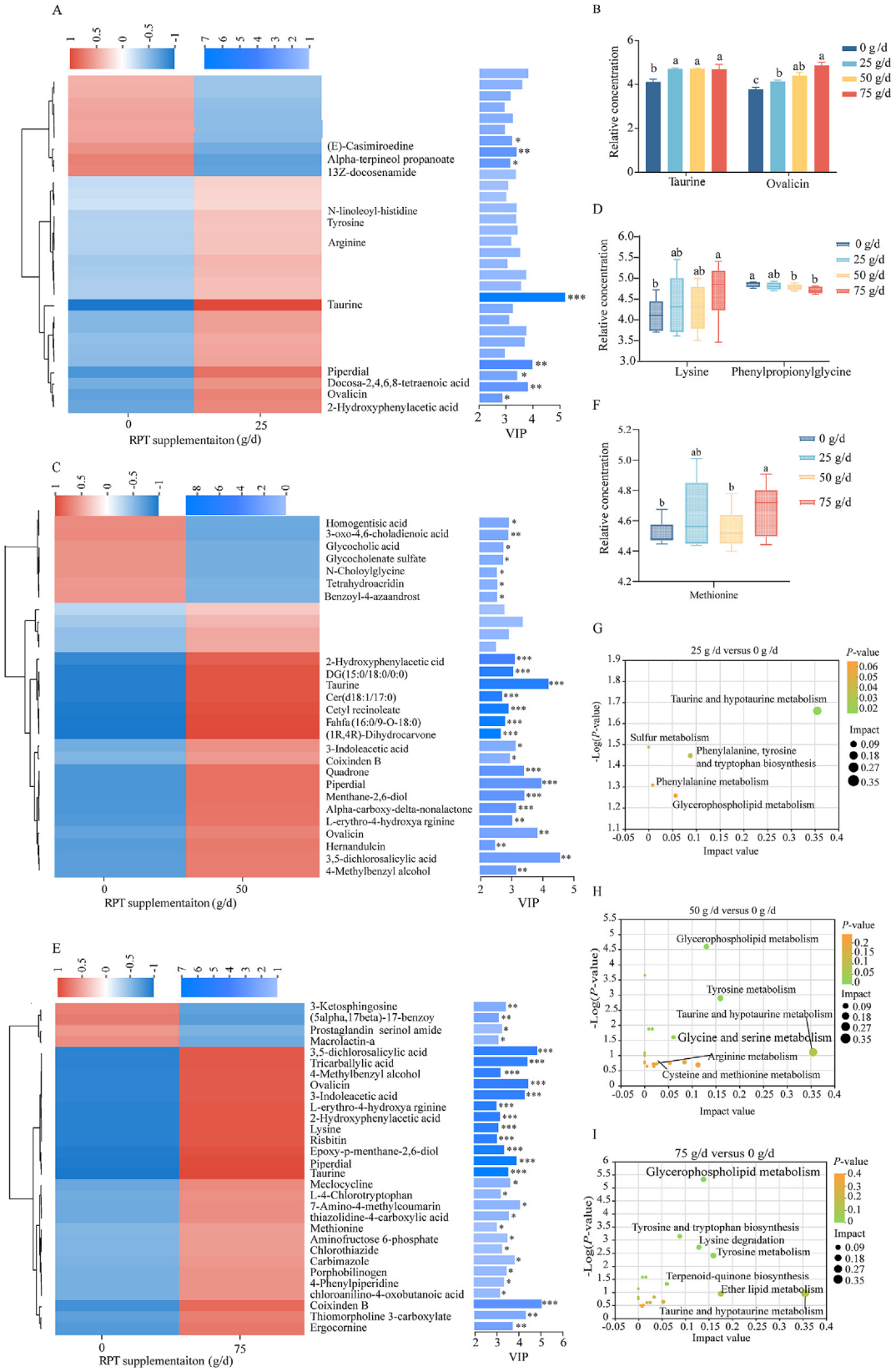

Fig. 1. Identification of the plasma metabolites and metabolite pathways as affected by rumen-protected taurine (RPT). (A) The heatmaps of the metabolites (VIP > 1, $P < 0.05$) as affected by supplementing RPTat 25 g/d. (B) The effect of RPT on plasma taurine and ovalicin. (C) The heatmaps of the metabolites (VIP > 1, $P < 0.05$) as affected by supplementing with 50 g RPT/d. (D) The effect of RPT on plasma lysine and phenylpropionylglycine. (E) The heatmaps of the metabolites (VIP > 1, $P < 0.05$) as affected by supplementing RPT at 75 g/d. (F) The effect of RPT on plasma methionine. (G to I) The pathways of the differential metabolites as affected by supplementing with RPT at 25, 50 and 75 g/d, respectively, in which the size of the bubbles represents the pathway impact value, the color of the bubbles represents the *P*-value (−lg *P*) of the metabolic pathway in enrichment analysis. The bars with different letters (a, b, c) in Fig. B, D and F represent significant difference ($P < 0.05$). The bars with asterisks in Fig. A, C and E indicate significant difference (* represents $P < 0.05$; ** represents $P < 0.01$; *** represents $P < 0.001$). VIP = variable importance projection.

groups, suggesting that taurine was the principal factor for the sample separation.

The DEGs and their functions were analyzed among different treatments. A total of 624 DEGs were detected, of which 287, 149 and 188 (Fig. 2A) were in the treatment groups supplemented with 25, 50 and 75 g RPT/d, respectively, compared to the control group.

To characterize the differential expressions in detail, the trend analysis was performed to explore the expression patterns of all DEGs. A total of 20 profiles were clustered, of which 5 profiles, i.e. 5, 14, 15, 17 and 19, showed significant enrichment, as shown in the color blocks (Fig. 2B). Supplementation with RPT linearly upregulated the expressions of the genes in profiles 19 and 17. Among

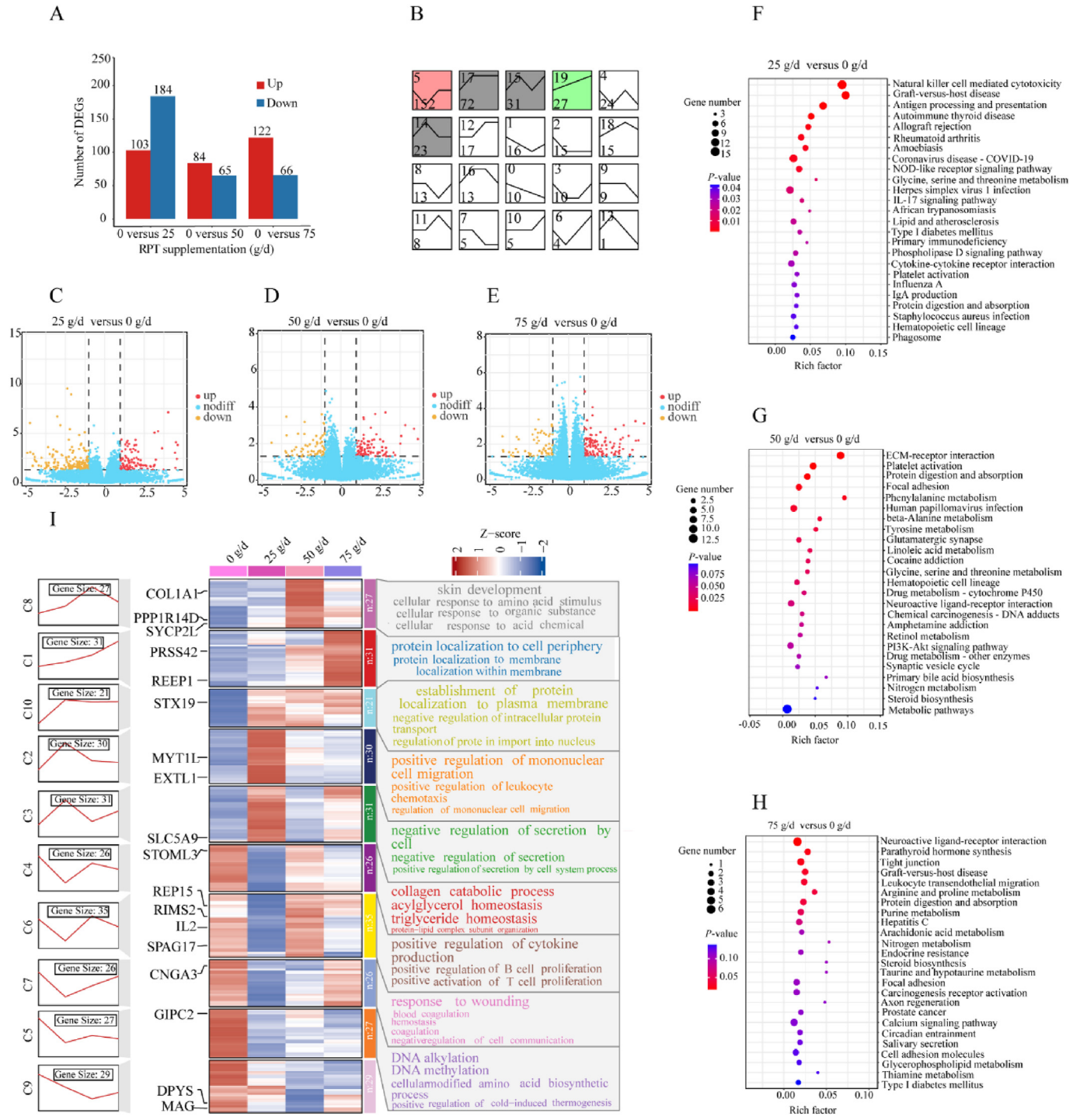

Fig. 2. Analysis of the whole-blood cells transcriptomes. (A) The number of the differentially expressed genes (DEGs) as affected by supplementing with rumen-protected taurine (RPT) at 25, 50 and 75 g/d, respectively. (B) The trend analysis of the DEGs. The colored-profiles represent the significantly altered gene expression, while the profiles with the same color represent the similar trends of gene expression. (C to E) The volcano plots of the whole-blood transcriptome as affected by supplementing with RPT at 25, 50 and 75 g RPT/d, respectively. Yellow, red and blue circles refer to up-regulated, down-regulated, and unchanged genes, respectively. (F to H) The bubble diagrams of the significant KEGG pathways of DEGs as affected by supplementing with RPT at 25, 50 and 75 g/d, respectively. (I) The transcript expression and the cluster enrichment.

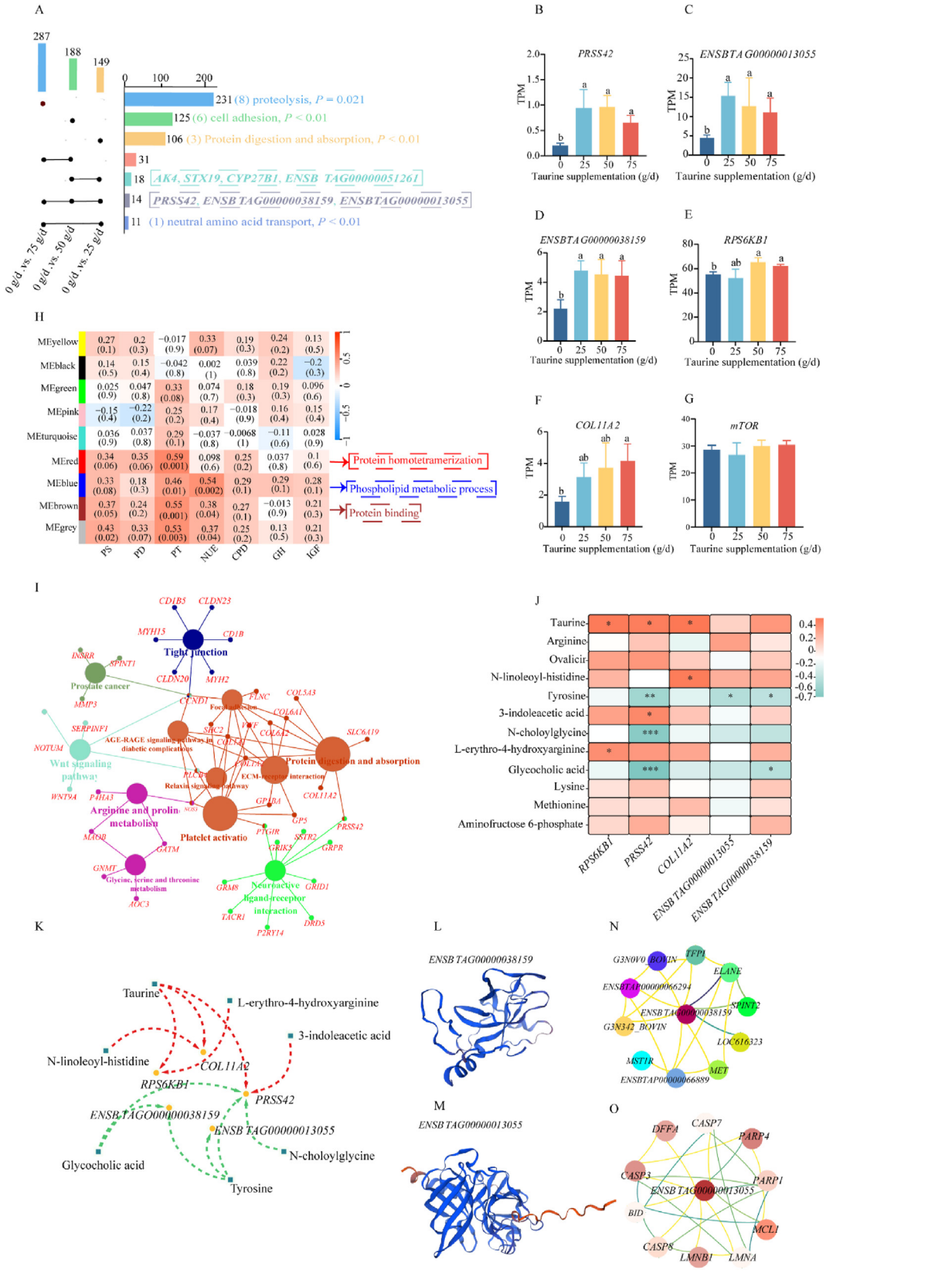

Fig. 3. The weighted gene co-expression network analysis (WGCNA), marker gene screening and their correlation with plasma differential metabolites. (A) The enriched gene ontology biological process (GO-BP) terms and the *P*-values in common and unique genes among different comparison gene sets. The dotted boxes show the genes for the corresponding intersection. (B to G) Effects of rumen-protected taurine (RPT) on marker gene expression. TPM = transcripts per kilobase of exon model per million mapped reads. The bars with different letters (a, b, c) in Fig. B, D and F indicate significant difference at $P < 0.05$. (H) The WGCNA reveals the relationships between the module gene expression and the parameters of protein metabolism, in which the values in the squares represent the correlation coefficient, the values in the brackets represent the *P*-values. PS = protein synthesis; PD = protein degradation; PT = protein turnover; NUE = nitrogen utilization efficiency; CPD = crude protein digestibility. Growth hormone (GH) and insulin-like growth factor-1

these DEGs, supplementing with 25, 50 and 75 g RPT/d upregulated 184, 65, and 66 genes and downregulated 103, 84, and 122 genes, respectively (Fig. 2C–E).

The KOBAS (http://kobas.cbi.pku.edu.cn/genelist/) was used to determine the functions of the DEGs with a cutoff of $P < 0.05$. The KEGG analysis indicates that supplementing with 25, 50 and 75 g RPT/d enriched the protein digestion and absorption pathway (Fig. 2F–H). In detail, supplementing with 25 g RPT/d enriched the pathways related to glycine, serine and threonine metabolism, and cytokine–cytokine receptor interaction (Fig. 2F); 50 g RPT/d enriched the pathways related to phenylalanine metabolism, beta-alanine metabolism, tyrosine metabolism, glycine, serine and threonine metabolism, and N metabolism (Fig. 2G); and 75 g RPT/d enriched the pathways involved in the arginine and proline metabolism, purine metabolism, arachidonic acid metabolism, taurine and hypotaurine metabolism, and N metabolism (Fig. 2H). The Biological Process (BP) revealed that the functions of the DEGs were associated with the protein localization and the cytokine regulation (Fig. 2I). The treemaps show that the functions of the DEGs were involved in the protein synthesis, regulation of protein import into nucleus, cellular response to amino acids stimulus, proteolysis, and the AMP metabolic process (Figs. S2B, C, D).

3.6. Candidate genes and weighted gene co-expression network

A total of 14 common DEGs were identified among the groups supplemented with 25, 50 and 75 g RPT/d and 18 common DEGs were identified in the groups supplemented with 50 and 75 g RPT/d (Fig. 3A). Among the 14 common DEGs, the expression of *PRSS42* gene related to protein degradation was upregulated with the increase of RPT dosage (Fig. 3B). Two novel candidate marker genes including *ENSBTAG00000013055* (Fig. 3C) and *ENSBTAG00000038159* (Fig. 3D) were identified by intersecting the common DEGs among the linearly upregulated profiles of 5, 17, 19 and 18. To further elucidate the mechanism through which taurine modulated the gene expression and subsequently the body protein synthesis and degradation, the variance analysis and the post hoc multiple comparisons of SAS 9.3 (SAS Inst. Inc., Cary, NC, USA) were used to assess whether taurine regulated the expression levels of the protein synthesis-related genes including *mTOR*, *RPS6KB1*, *AKT1*, *EIF4EBP1*, and *IRS1*, as well as the protein degradation-related genes including *UBA1* and *UBA6*. The results indicated that supplementing with 50 and 75 g RPT/d upregulated the expressions of *RPS6KB1* (Fig. 3E) and *COL1A2* (Fig. 3F) in the whole-blood cells of the steers, whereas it did not affect the expressions of mTOR (Fig. 3G), *AKT1*, *EIF4EBP1*, *IRS1*, *UBA1*, and *UBA6*. The WGCNA was performed to investigate the co-expression networks of the DEGs related to the whole-body protein turnover, protein synthesis and NUE. A total of 9 modules were identified based on similar gene expression patterns (Fig. 3H). According to the module-trait correlation heatmap, the red, blue, brown and grey modules were identified with close correlation relationships to the body protein synthesis, protein accretion and NUE, respectively. These modules were then extracted and enriched by the GO term. The red, blue and grey modules were primarily related to the protein homotetramerization, phospholipid metabolic process, and protein binding, respectively. Based on the WGCNA and the intersections of the DEGs in the red, blue, brown, and grey modules, it was found that supplementing with 75 g RPT/d upregulated the expression of a candidate marker gene *COL11A2*. The ClueGO analysis indicated that *PRSS42* and *COL1A2* were enriched in the pathway of protein digestion and absorption (Fig. 3I). Overall, a total of five candidate marker genes were identified, including *RPS6KB1*, *PRSS42*, *ENSBTAG00000013055*, *ENSBTAG00000038159* and *COL1A2*.

3.7. Correlations between the marker genes and the plasma metabolites

The Spearman correlation coefficients were calculated between the marker genes and the differential metabolites in plasma. *RPS6KB1* was found to be positively correlated with the plasma concentrations of taurine and L-erythro-4-hydroxyarginine (Fig. 3J and K). *PRSS42* was negatively correlated with the plasma concentrations of tyrosine, N-choloylglycine, glycocholic acid, and positively correlated with the plasma concentration of taurine (Fig. 3J and K). *COL1A2* was positively correlated with the plasma concentrations of N-linoleoyl-histidine and taurine. *ENSBTAG00000013055* was negatively correlated with the plasma concentration of tyrosine, while *ENSBTAG00000038159* was negatively correlated with the plasma concentrations of tyrosine and glycocholic acid (Fig. 3J and K).

3.8. Prediction of the structures and functions of the proteins encoded by novel genes

The gene's coding sequences analysis (Table S1) revealed that the protein encoded by *ENSBTAG00000013055* was an unstable protein whereas the protein encoded by *ENSBTAG00000038159* was a stable one. The prediction of the subcellular localization of the proteins encoded by *ENSBTAG00000013055* and *ENSBTAG00000038159* indicated that the two proteins predominantly resided in the extracellular matrix. The prediction of the protein structures suggested that the secondary and tertiary structures of the proteins encoded by *ENSBTAG00000038159* (Fig. 3L) and *ENSBTAG00000013055* (Fig. 3M) were primarily composed of α-helices and random coils. The protein interaction analysis revealed that the protein encoded by *ENSBTAG00000038159* was closely correlated with the proteins including TFP1, SPINT2, ELANE, MST1R, and MET (Fig. 3M), while the protein encoded by *ENSBTAG00000013055* was closely correlated with the proteins including DFFA, PAPR4, LMNB1, CASP3, CASP7, PARP1, BID, CASP8, and LMNA (Fig. 3O).

4. Discussion

Previous studies showed that taurine supplementation increased the ADG, decreased the feed/gain ratio (Zeng et al., 2009), improved the growth performance of broiler chickens (Lee et al., 2004), and enhanced the growth and health of carp fry (Abdel-Tawwab and Monier, 2018). The present experiment showed that dietary supplementation with RPT increased the ADG of steers. It

(IGF-1) represent plasma growth hormone and insulin-like growth factor-1, respectively. Three key WGCNA modules and partially enriched GO-BP terms are listed on the right side. (I) Visualization of gene ontology (GO) enrichment profiles from differentially expression genes (DEGs) using Cytoscape software based on the network analysis of ClueGO/CluePedia. (J) The heatmap for the correlations of the marker genes and the differential metabolites of the plasma, the positive correlations are displayed in red and the negative correlations in blue color. The color intensity is proportional to the correlation coefficients. The correlations were considered statistically significant at $P < 0.05$ (*), $P < 0.01$ (**) and $P < 0.001$ (***). (K) The network plots for the whole-blood cells expressed genes and metabolites. The blue squares represent the metabolites. The orange circles represent the genes. The dotted lines represent the regulatory relationships (red for positive and green for negative correlation). (L) The predicted tertiary structure of the protein encoded by *ENSBTAG00000038159* gene. (M) The predicted tertiary structure of the protein encoded by *ENSBTAG00000013055* gene. (N) The interaction network of the protein encoded by *ENSBTAG00000038159* gene with other proteins. (O) The interaction network of the protein encoded by *ENSBTAG00000013055* gene with other proteins.

should be noted that each period of the present experiment was only 20 days and the animals experienced intensive manipulations. Therefore, the impact of taurine on the ADG of steers was largely indicative rather than conclusive. These results were in agreement with previous studies conducted in broilers and fish. It was reported, however, that dietary supplementation with 20 and 40 g/d unprotected taurine did not affect the ADG of steers (Liu et al., 2023a). The discrepancy in the effects of taurine on steer ADG could be due to the high degradability of unprotected taurine in rumen fermentation (Zhang et al., 2023).

The results of the present experiment showed that supplementing with RPT linearly increased the NUE in steers. The impact could be attributed to the decreased fecal N excretion and increased body protein synthesis as affected by RPT supplementation. However, Liu et al. (2023a) reported that dietary supplementation with 20 and 40 g/d unprotected taurine did not affect the NUE in steers, which could also be attributed to the high degradability of unprotected taurine in the rumen (Zhang et al., 2023).

In the present experiment, supplementing with RPT improved the whole-body protein synthesis and degradation in steers. However, the effect of supplementing with RPT on protein synthesis was greater than that on protein degradation, hence resulting in an increase in whole-body protein accretion and protein turnover. The results indicated that supplementing with RPT regulated the protein metabolism and improved the whole-body protein turnover and accretion.

Growth hormone and IGF-1 are essential hormones that are associated with the body protein synthesis. Growth hormone can stimulate the IRS1/Akt and the mitogen-activated protein kinase (MAPK) pathways to facilitate protein synthesis (Consitt et al., 2017), and IGF-1 can activate the Akt-mTORC1 pathway and promote protein synthesis (Schiaffino and Mammucari, 2011). The results of the present experiment indicated that supplementing with 25, 50 and 75 g RPT/d linearly increased the plasma concentration of GH and quadratically affected the plasma concentration of IGF-1. The results of the present experiment were in line with Ikuyama et al. (1998) and Morshedi et al. (2022), who indicated that taurine was an effective stimulator of GH secretion in rats (Ikuyama et al., 1988), and taurine increased the blood concentration of IGF-1 in Asian seabass juvenile (*Lates calcarifer*) (Morshedi et al., 2022). These results suggest that the impact of taurine on the body protein synthesis, and consequently the whole-body protein turnover and accretion in steers could be through regulating the plasma concentration of GH and IGF-1. In addition, the results of the present experiment showed that supplementing with RPT did not affect the plasma concentrations of TP and GLB but decreased the plasma concentration of ALB. The results are consistent with Liu et al. (2023b), who reported a negative correlation between the serum taurine concentration and the serum ALB concentration in cats. However, the mechanism for the impact of taurine on the plasma parameters is unclear and needs further research.

The plasma AA concentration is one of the most important factors that mediates the protein synthesis both in liver and muscles (O'Connor et al., 2003). Lys is one of the limiting AA for protein synthesis in animals, and the covalent modification of Lys residues is a well-recognized regulatory mechanism for gene transcription (Zhao et al., 2010). The untargeted metabolomics profiling indicated that supplementing with 75 g RPT/d upregulated the plasma concentrations of taurine, lysine and methionine. The results suggested that Ttaurine regulates the whole-body protein synthesis possibly through modulation of AA metabolism in steers. Consistent with these results, supplementing with RPT upregulated three different kinds of plasma AA metabolites, including 3-indoleacetic acid, kynurenic acid and N-undecanoylglycine. Since 3-indoleacetic acid and kynurenic acid are the metabolites of tryptophan, and N-undecanoylglycine is the metabolite of glycine (Xiang et al., 2020), the impact of taurine on regulating the whole-body protein degradation should be partly through modulation of AA metabolism in steers. Meanwhile, the results showed that supplementing with RPT upregulated the plasma concentration of phenylpropionylglycine, which could modulate the target genes responsible for AA homeostasis upon binding to PPARα as its ligand (Peters et al., 2005). The KEGG enrichment analysis revealed that supplementing with RPT enriched the pathways of phenylalanine, tyrosine and tryptophan biosynthesis, lysine degradation, tyrosine metabolism, arginine and proline metabolism, PI3K-Akt signaling, N metabolism and proteolysis, which are all closely related to the protein synthesis and degradation in animals. Overall, the results indicated that supplementing with RPT regulated the whole-body protein turnover through modulating the plasma AA and its metabolites, even though the key factors were unclear.

Whole-body protein turnover is a result of the physiological processes which involve many genes. It was reported that taurine improved the protein translation in the mitochondrial network through conjugating the tRNA of mitochondria without being incorporated into protein in human and animals (Suzuki et al., 2002). However, no reports are available on the impact of taurine on the regulation of whole-body protein turnover. In the animal body, protein synthesis is mainly initiated by the mTORC1 phosphorylation, which is activated by factor 4E binding protein (*4E-BP*) and p70 S6 kinase 1 (*RPS6KB1*) (Liu and Sabatini, 2020), and similarly, as reported by Guillet et al., *RPS6KB1* is associated with the muscle protein synthesis (Guillet et al., 2004) while 80% to 90% of body protein is degraded by the ubiquitin proteasome system (Lilienbaum, 2013). The whole blood-cells transcriptome of the present experiment revealed that supplementation with RPT enhanced the expression of *RPS6KB1*, suggesting that taurine increased body protein synthesis through upregulation of *RPS6KB1* expression.

A great variety of proteases, such as cathepsins, proteasomes, and most exopeptidases modulate body protein metabolism (Turk et al., 2012). The results of this experiment showed that supplementation with RPT upregulated the expression of serine protease 42 (*PRSS42*). A study in humans showed that after 45 min of acute exercise at 70% of maximal oxygen uptake, *PRSS42* was upregulated in muscle protein recovery (Pourteymour et al., 2017), suggesting that this gene is related to muscle protein synthesis. However, no direct reports are available on the impact of *PRSS42* on the body protein metabolism. In the present experiment, the integration analysis of the DEGs expression profile with the plasma metabolites indicated that *PRSS42* was positively correlated with the plasma 3-indoleacetic acid, suggesting that *PRSS42* regulateed the tryptophan metabolism. Hence, *PRSS42* could possibly be a potential gene for regulating body protein metabolism. The results of the present experiment also showed that supplementing with RPT upregulated the expression of *COL1A2*, which is a member of the collagen family. Because *COL1A2* is involved in epithelial−mesenchymal transition and regulates a variety of cellular processes, including cell proliferation, metastasis (Li et al., 2022), protein digestion and absorption (Adhikari et al., 2022), the impact of taurine on protein metabolism could be partly through regulating the expression of *COL1A2*. The present experiment indicated that supplementing with 50 and 75 g RPT/d upregulated the expression of two novel genes, including *ENSBTAG00000013055* and *ENSBTAG00000038159*. Based on the prediction of the structures and the physicochemical properties of the encoded proteins of the two genes, it was found that *ENSBTAG00000013055* encoded protein was closely related to CASP3, which is an important protein for the degradation of myofibrillar proteins (Smuder et al., 2010), and *ENSBTAG00000038159* encoded protein was closely related to ELANE,

which forms a subfamily of serine proteases that hydrolyze many proteins, particularly elastin (González et al., 2016). Additionally, the present experiment indicated that the expressions of *ENSBTAG00000013055* and *ENSBTAG00000038159* were negatively correlated with the plasma concentration of tyrosine, which is an essential AA for protein synthesis in animal body. The results indicated that supplementing with RPT improved the body protein synthesis by upregulating the expressions of *RPS6KB1*, *PRSS42* and *COL1A2* while enhanced the body protein degradation by upregulating the expressions of *ENSBTAG00000013055* and *ENSBTAG00000038159*, resulting in improved whole-body protein turnover and protein accretion. The impact of taurine on improving whole-body protein turnover and renewal suggest supplementing with RPT has the potential to enhance the cell viability and improve the growth and health status in animals.

5. Conclusion

Supplementing with RPT improved the protein synthesis, protein degradation and consequently enhanced the whole-body protein turnover and protein accretion in steers. The impact of supplementing with RPT on the protein metabolism could be attributed to the effects of taurine on modifying the plasma metabolome and whole-blood cells transcriptome and increasing the plasma concentrations of IGF-1 and GH. Two novel genes including *ENSBTAG00000013055* and *ENSBTAG00000038159* were found to be related to body protein degradation in steers. The present experiment indicated that supplementing with taurine may enhance the cell viability and improve the growth and health status in animals. Further research is needed for a wide range of animal species and humans to verify the effects of taurine on the body protein renewal and to elucidate the functions of the novel proteins encoded by *ENSBTAG00000013055* and *ENSBTAG00000038159* in animals.

Author contributions

Shuo Zhang: Investigation, Formal analysis, Data Curation, Writing – Original draft preparation. **Jinming Hu:** Investigation, Formal analysis. **Yufeng Liu:** Formal analysis. **Xu Shen:** Formal analysis. **Cheng Liu:** Formal analysis. **Long Cheng:** Formal analysis. **Mengmeng Li:** Writing – Review & Editing. **Guangyong Zhao:** Conceptualization, Methodology, Supervision, Writing – Review & Editing, Project Administration.

Data availability

The raw transcriptomic data of the whole-blood cells were deposited in NCBI (PRJNA1049616).

Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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Appendix supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aninu.2024.07.002.

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